A COMPARATIVE STUDY OF INTERNATIONAL CLASSIFICATION FOR LUNG CANCER AND WHO CLASSIFICATION IN HISTOLOGICAL DIAGNOSIS OF LUNG CANCER IN SMALL BIOPSIES

Dissertation submitted in

partial fulfilment of the requirements for the degree of

M.D. (PATHOLOGY)

BRANCH - III

GOSCHEN INSTITUTE OF PATHOLOGY AND ELECTRON MICROSCOPY

MADRAS MEDICAL COLLEGE

CHENNAI – 600 003

THE TAMIL NADU

DR. M.G.R. MEDICAL UNIVERSITY

CHENNAI

APRIL 2015

CERTIFICATE

This is to certify that this Dissertation entitled **"A COMPARATIVE STUDY OF INTERNATIONAL CLASSIFICATION FOR LUNG CANCER AND WHO CLASSIFICATION IN HISTOLOGICAL DIAGNOSIS OF LUNG CANCER IN SMALL BIOPSIES**" is the bonafide original work of **Dr. NITHYA. I**, in partial fulfillment of the requirement for M.D., (Branch III) in Pathology examination of the Tamilnadu Dr.M.G.R Medical University to be held in April 2015.

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DECLARATION

I, **Dr. Nithya .I,** solemnly declare that the dissertation titled "**A COMPARATIVE STUDY OF INTERNATIONAL CLASSIFICATION FOR LUNG CANCER AND WHO CLASSIFICATION IN HISTOLOGICAL DIAGNOSIS OF LUNG CANCER IN SMALL BIOPSIES**" is the bonafide work done by me at Institute of Pathology, Madras Medical College under the expert guidance and supervision of **Prof. Dr. RAMAMOORTHY,** M.D., Professor of Pathology,Institute of pathology, Madras Medical College. The dissertation is submitted to the Tamilnadu Dr. M.G.R Medical University towards partial fulfillment of requirement for the award of M.D., Degree (Branch III) in Pathology.

Place: Chennai

Date: **Dr. NITHYA.I**

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I dedicated the entire thesis work to my late son, B.Siddharth, our bundle of joy, alive in our heart. He continues to live in this world, till our heartbeats.

INSTITUTIONAL ETHICS COMMITTEE
MADRAS MEDICAL COLLEGE, CHENNAI-3

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CERTIFICATE OF APPROVAL

To
Dr. I. Nithya,
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Dear Dr. I. Nithya,

The Institutional Ethics Committee of Madras Medical College, reviewed and discussed your application for approval of the proposal entitled **"Comparative study of international classification for lung cancer and WHO classification in histological diagnosis of lung cancer in small biopsies"** No.23022014

The following members of Ethics Committee were present in the meeting held on 04.02.2014 conducted at Madras Medical College, Chennai-3.

1.	Dr. G. Sivakumar, MS FICS FAIS	-- Chairperson
2.	Prof. Kalaiselvi, MD Prof. of Pharmacology, MMC, Ch-3	-- Member Secretary
3.	Prof. Dr. K.Ramadevi, Director i/c, Instt. of Biochemistry, Chennai	-- Member
4.	Dr. Geetha Devadoss, Associate Professor of Pathology, MMC, Ch-3.	-- Member
5.	Prof. Dr. Sivasubramanian, I/c Director, Institute of Internal Medicine, MMC, Ch-3.	-- Member
6.	Thiru. S. Govindasamy, BABL	-- Lawyer
7.	Tmt. Arnold Saulina, MA MSW	-- Social Scientist

We approve the proposal to be conducted in its presented form.

Sd/Chairman & Other Members

The Institutional Ethics Committee expects to be informed about the progress of the study, and SAE occurring in the course of the study, any changes in the protocol and patients information / informed consent and asks to be provided a copy of the final report.

19/6/14

Member Secretary, Ethics Committee

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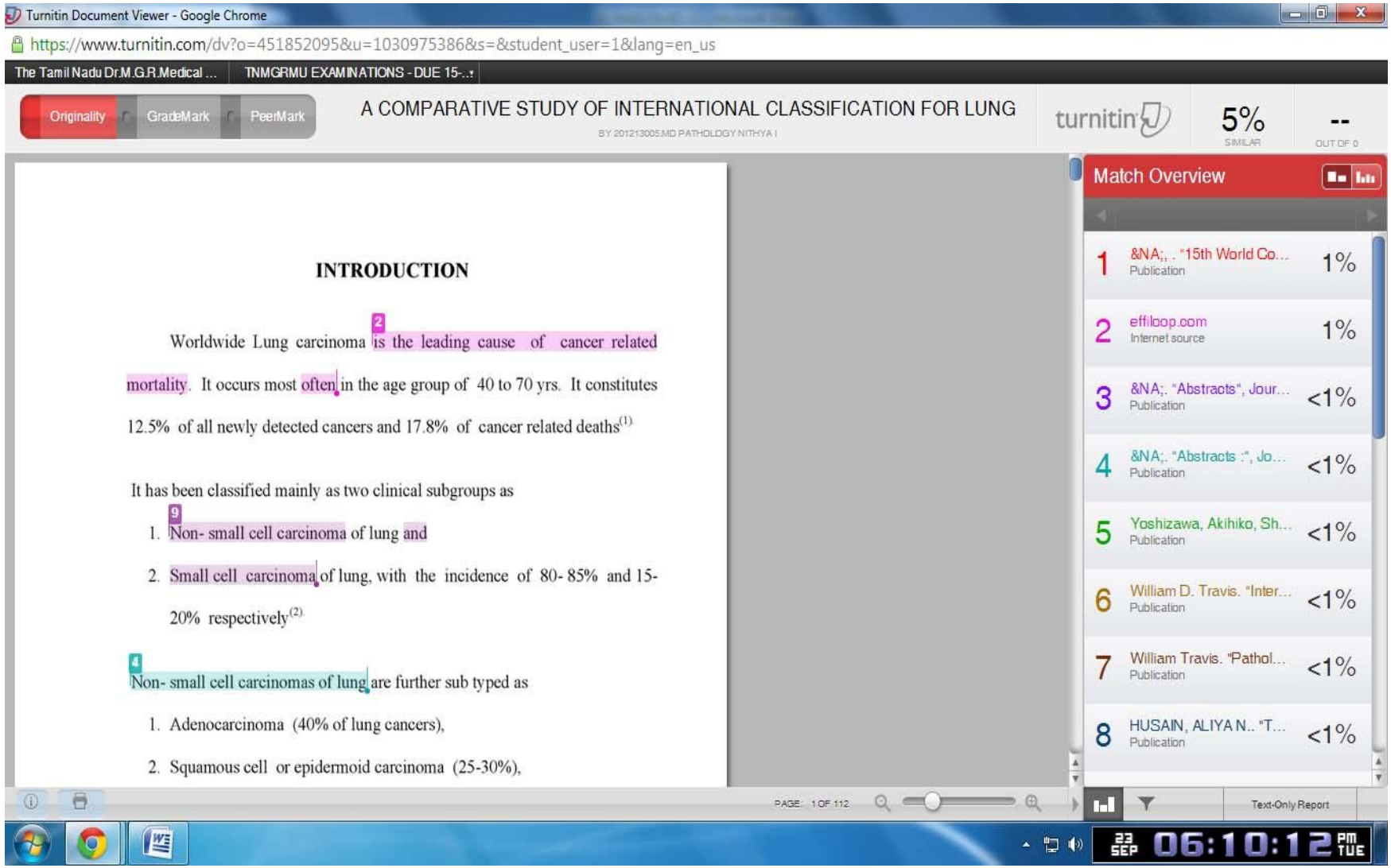
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INTRODUCTION
Worldwide Lung carcinoma is the leading cause of cancer related mortality. It occurs most often in the age group of 40 to 70 yrs. It constitutes 12.5% of all newly detected cancers and 17.8% of cancer related deaths(1).
It has been classified mainly as two clinical subgroups as
1. Non- small cell carcinoma of lung and
2. Small cell carcinoma of lung, with the incidence of 80- 85% and 15-20% respectively(2).
Non- small cell carcinomas of lung are further sub typed as
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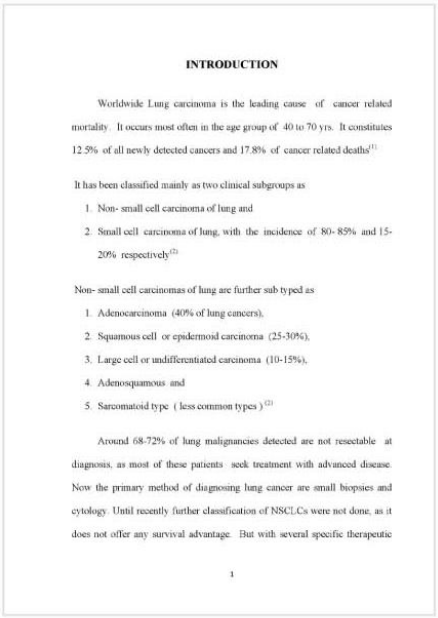

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3. Large cell or undifferentiated carcinoma (10-15%),
4. Adenosquamous and
5. Sarcomatoid type (less common types) [2]

Around 68-72% of lung malignancies detected are not resectable at diagnosis, as most of these patients seek treatment with advanced disease. Now the primary method of diagnosing lung cancer are small biopsies and cytology. Until recently further classification of NSCLCs were not done, as it does not offer any survival advantage. But with several specific therapeutic

1

ABBREVIATIONS

SCC	:	Squamous cell carcinoma
ADC	:	Adenocarcinoma
EGFR	:	Epidermal growth factor receptor
WHO	:	World Health Organisation
IASLC/ATS/ERS	:	International Association for the Study of Lung Cancer/American Thoracic Society/European Respiratory Society
NSCLC	:	Non Small Cell Lung Carcinoma
NSCLC-NOS	:	Non Small Cell Lung Carcinoma-not otherwise specified
TTF-1	:	Thyroid Transcription Factor-1
AB/PAS	:	Alcian Blue-Periodic Acid Schiff Reagent
IHC	:	Immunohistochemistry
H & E	:	Hematoxylin & Eosin
CIS	:	Carcinoma in situ
BAC	:	Bronchoalveolar Carcinoma
AIS	:	Adenocarcinoma Insitu
MIA	:	Minimally Invasive Adenocarcinoma

CONTENTS

A COMPARATIVE STUDY OF INTERNATIONAL CLASSIFICATION FOR LUNG CANCER AND WHO CLASSIFICATION IN HISTOLOGICAL DIAGNOSIS OF LUNG CANCER IN SMALL BIOPSIES

ABSTRACT

BACKGROUND : Lung cancer is a highly aggressive malignancy causing high morbidity and mortality. An increasing incidence of lung cancer has been observed in India. Currently, the classification of lung carcinoma has gone beyond small cell lung carcinoma and non‑small cell lung carcinoma (NSCLC). Precise subtyping of poorly differentiated NSCLC into adenocarcinoma and squamous cell carcinoma has a direct impact on patient management and prognosis.70% of lung cancers are unresectable, as patients present in advanced stages. Hence small biopsy and cytology specimens are the primary method of diagnosis for the majority of lung cancers.Also, prior 2004WHO classifications primarily addressed resection specimens, they did not propose standardized terminology and criteria for small biopsies and cytology. Hence this new 2011 IASLC classification provides for the first time a proposed set of terms and criteria for all major histologic types of lung cancer in small biopsies and cytology.

AIMS AND OBJECTIVES: To classify lung cancer according to International classification based on morphology, special stains and IHC in small biopsies and compare the same with previous WHO classification. And finally to determine the diagnostic supremacy of one classification over the other and its therapeutic implications.

MATERIALS AND METHODS: 151 cases Paraffin sections of small biopsy samples diagnosed as Non small cell lung carcioma will be subjected to routine H&E staining and supplemented to special stain for mucin (alcian blue/PAS) and IHC markers p40(marker of SCC) and TTF1(marker of adenocarcinoma)

RESULTS : Of the total 151 cases studied on morphological basis, 121 Cases were diagnosed as adenocarcinoma and squamous cell carcinoma. The remaining 30 cases diagnosed as NSCLC-NOS. In this study, According to IASLC/ATS/ERS, the percentage of NSCLC – NOS was minimised with the use of alcian blue/PAS and the IHC markers p40 and TTF 1, from 19.86% to 1.86%

CONCLUSION : According to this study we conclude that multidisciplinary International Classification For Lung Cancer is superior to 2004 WHO classification in terms of diagnostic, therapeutic and prognostic implications.

INTRODUCTION

Worldwide Lung carcinoma is the leading cause of cancer related mortality. It occurs most often in the age group of 40 to 70 yrs. It constitutes 12.5% of all newly detected cancers and 17.8% of cancer related deaths[1].

It has been classified mainly as two clinical subgroups as

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5. Sarcomatoid type (less common types) [2]

Around 68-72% of lung malignancies detected are not resectable at diagnosis, as most of these patients seek treatment with advanced disease. Now the primary method of diagnosing lung cancer are small biopsies and cytology. Until recently further classification of NSCLCs were not done, as it does not offer any survival advantage. But with several specific therapeutic

options available today for patients with squamous, adenocarcinoma or NSCLC-NOS, further sub-classification of NSCLC based on small tissue biopsy and cytology using tumour markers and special stains have become necessary. [3,4]

WHO classification of lung cancer(2004) is based on resection specimens and primarily addressed only them. This classification did not propose any standardised criterias for small biopsies and cytology in lung cancer[1]. But the new multidisciplinary classification proposed by INTERNATIONAL ASSOCIATION FOR THE STUDY OF LUNG CANCER / AMERICAN THORACIC SOCIETY / EUROPEAN RESPIRATORY SOCIETY (IASLC/ATS/ERS 2011) provides, for the first time, certain terms and criteria for all major types of lung cancer based on cytology and small biopsy.

This study is aimed at classifying the lung cancer with small biopsies using special stains and immuno-histochemistry (IHC) markers according to IASLC/ATS/ERS proposed new international multidisciplinary classification, and to compare with previous standardised 2004 WHO classification and its therapeutic implications.

Aims and Objectives

AIMS AND OBJECTIVES

- To classify lung cancer in accordance with the new classification proposed by International association for the study of lung cancer/American thoracic society/European respiratory society (IASLC/ATS/ERS 2011) .

- To compare with the previous 2004 WHO classification of lung tumours.

- To determine the diagnostic supremacy of one classification over the other.

- To assess the therapeutic implications of recent classification.

Review of Literature

REVIEW OF LITERATURE

Epidemiology:

Worldwide carcinoma of lung is the most common cancer for several decades. In 2012 ,it is estimated to be 1.8 million new cases of lung carcinoma detected.this constitutes about 12.9% of all cancer cases. among these 58% of cases occured in less developed countries. Lung cancer is the most common malignancy among men worldwide (1.2million cases) with highest age standardised rates in east and central Europe (53.5 per 1lakh population) and eastern Asia (50.4 per 1lakh population). Low incidence rates are observed in western and middle Africa (1.7 & 2 respectively per 1lakh population).

In women, the incidence of lung cancer are generally low. the geographical pattern of lung cancer is little different and it mainly reflects the different historical exposure to tobacco smoke. Regarding incidence of this disease, the highest recorded are in north America (33.8%) and northern Europe (23.7%) with relatively high rate in Asia (19.2%) and the lowest incidence in middle and western Africa. Lung cancer remains the commonest cause of cancer related mortality worldwide and it is estimated nearly 1 in every 5cases of deaths are due to lung cancer (about 1.59 million deaths accounting for 19.4% of all deaths). Because of the high fatality and relative

risk of survival variability in different world regions, the geographical pattern in mortality from lung cancer closely follows those with incidence [5].

In India lung cancer remains the most common and severe form of cancer among males. It accounts for 10.9% of cancer cases and 13% of cancer related deaths .Its incidence is low among Indian women.[5]

Etiology and Pathogenesis:

Tobocco smoke:

Among the risk factors, Smoking is the leading cause for lung cancer. At least 80% of lung malignancy related deaths are due to tobacco smoke. lung cancer occur mostly (86%) in active smokers and those quit smoking recently. There has been proven statistical association between the incidence of lung malignancy and smoking and it depends on

1. The duration
2. The quantity of cigarettes and
3. Depth of inhalation of smoke

Smokers are at increased risk(10 fold) and heavy smokers (> 40 cigarettes per day) have sixty times more risk for lung cancer ,when compared to non smokers,. susceptibility to tobacco carcinogens is more in women when compared to men . Despite all this only 11% of heavy smokers develop lung cancer in their lifetime. This shows that additionally some

genetic factors also plays a role. Studies shows that industrial exposure to tobacco and second hand smoke also contains many human carcinogens.[6,7,8]

Clinical evidence is obtained from habitual smokers through observation of all histological changes in their respiratory tract epithelium. Linear correlation is observed between the intensity of tobacco smoking and the appearance of changes in epithelial lining of lung. This change begins with squamous metaplasia, CIS and then invasive carcinoma, in that sequence[9].

Radon:

It is a gas which occurs naturally and is obtained from breakdown of substances like uranium present in earth . Radon is identified as second leading risk factor for lung cancer in united states.[8,10,11]

Industrial hazards:

Exposure to occupational hazards accounts for about 5-10% of lung carcinoma cases, in industrialised countries. industrial exposure such as ionising radiation, , asbestos, arsenic, cadmium, beryllium, vinyl chloride , silica ,nickel compounds, mustard gas ,chromium, coal products, diesel exhaust etc increases the risk of lung cancer . Exposure to ionising radiation is a moderate risk factor for this type of malignancy as in patients treated with radiotheraphy and in atomic bomb survivors.

Asbestos:

Asbestos is an important risk factor for Lung cancer and the most common cancer in peoples exposed to asbestos is lung cancer. Smoking in Asbestos workers have 50 to 90 times increased risk than do non-smokers.[12,13]

Air pollution:

Air pollution increases the risk for lung cancer. It may be due to both Outdoor or Indoor cause. Worldwide it constitutes about 5% of all lung cancer cases. Indoor air pollution may be responsible for increased risk among non smoking women in Asia including some parts of china. This risk is highest among women living in poorly ventilated homes where wood, coal and other solid fuels are burnt regularly. Fumes from unrefined vegetable oils also increases the risk.[11,12,14]

Radiation therapy:

Radiation therapy to the chest as treatment for other malignancies (eg: as for Hodgkins disease or Carcinoma breast etc) are at higher risk for lung cancer.[14]

Geographical location:

People living in certain areas of south-America and south-Asia with high arsenic levels in drinking water are at increased risk.[14]

Family history:

Inheritance of certain DNA changes on a specific chromosomes are at increased risk for carcinoma of lung.

Dietary supplements:

Studies showed that smokers taking beta-carotene supplements develops lung cancer with increasing frequency. The possible role of other vitamins in decreasing the risk of lung malignancy, is not promising so for.[14]

Molecular genetics:

Exposure to risk factors will cause genetic alterations in lining epithelial cells of lung, which accumulate and lead to malignancy. Some molecular lesions are common for both clinical types (small cell and non small cell lung cancer), but some are very specific.

Genes frequently associated with lung cancer are ,

- **Dominant oncogenes** such as c-MYC, EGFR, c-MET, KRAS and c-KIT[15] are inactivated and / or deleted.
- **Tumour suppressor genes** such as p53, p16(INK4a) , RB1 and many loci on chromosome 3p.

So far the identified genetic factors involved in lung cancer are ,

1. In small cell carcinoma, C-KIT (~40-70%), 3p (100%) , p53(~90%), BCL2(70-90%), RB(~90%) MYCN and MYCL (20-30%) are commonly involved.

2. In non-small cell lung carcinoma KRAS(10-15%), p53(50%), p16INK4a(70%), EGFR(25%) , ALK(5%) are involved [15-18]

ANATOMY AND HISTOLOGY OF LUNGS

GENERAL CONSIDERATIONS:

The lungs are paired intra thoracic organs that in turn are divided into lobes. On right side it is divided into three as upper, middle and lower lobes. On left side into two as upper and lower lobes. There is a rudimentary appendage arising from upper lobe of left lung called lingula which is the analog of the middle lobe on the right side. The lobes are divided by fissures and each have their own pleura investments. The lobes were further subdivided into broncho pulmonary segments. In an normal individual there were approximately 20 generations extending from trachea upto respiratory bronchioles. Airways are defined as follows

- Trachea : Major Cartilagenous airway,
- Bronchi : Cartilagenous airway and are usually greater than 1mm .
- Bronchioles : These airways lack cartilage and are usually less than 1mm.
- Non-respiratory bronchioles : Represent all bronchioles proximal to respiratory bronchiole.
- Terminal bronchiole: The last non respiratory bronchiole is termed as terminal bronchiole.
- Respiratory bronchioles: Airways where gaseous exchange takes place and are lined with alveoli in their walls.

HISTOLOGY:

The respiratory tract is lined by pseudo stratified columnar epithelium composed primarily of ciliated columnar cells interspread with mucous cells, and less number of brush cells, neuro-endrocrine cells, and migrated inflammatory cells. The height of the pseudo stratified epithelium decreases progressively towards the periphery of lung. The importance of this segmental anatomy for pathologist, radiologist, bronchoscopy specialist is in clearly defining the lesion location.[18]

ORIGIN:

The site of origin for lung cancer refers to the type of tissue from which the cancer cells develops [18,19.] Usually lung cancer is categorized by its site of origin into hilar and peripheral types, as these structures from where the disease originates are different . The majority of the early lung cancers arising in hilar regions are squamous cell carcinoma, whereas those early stage cancers arising in the peripheral areas of lung are adenocarcinomas.[19]. Adenocarcinomas usually originates in glandular tissue whereas squamous cell carcinoma originates in the tissue that lines the organs and tubes of the lungs called epithelial tissues [20]. NSCLCs such as adenocarcinomas and large cell lung carcinoma are located typically in the peripheral areas of lungs and can present as either solitary nodule or masses [19]. Squamous cell carcinoma and small cell carcinoma are normally found to arise in the central portions of the lung and may be misdiagnosed as collapsed lung (Atelectasis) or pneumonia[19]. Small cell carcinoma are usually located in the main bronchi. this type of malignancy appears to originates from the Kulchitsky cells, which in turn is a component of the bronchial epithelium.[19].

Precancerous lesions of lung:

According to the recently publicised tumour classification system of WHO there are three well defined precancerous lesions of lung as

- **Squamous dysplasia / carcinoma in-situ:**

This represents the precursors of SCC of lung.

- **Atypical Adenomatous hyperplasia (AAH) :**

This adenomatous hyperplasia represents the precursor lesion for adenocarcinoma . it usually seen in peripheral lesions and

- **Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia:**

This may progresses to carcinoids. Other possible pre-neoplastic lesions are Squamous metaplasia (which progresses to squamous dysplasia,carcinoma in situ), Adenomatous hyperplasia (precursor to AAH), Basal cell hyperplasia, pulmonary fibrosis, angiogenic squamous dysplasia , etc.[21]

No precancerous lesion is identified for small cell carcinoma so far. But sometimes precursors of NSCLSs such as squamous dysplasia or carcinoma in-situ be seen in the nearby airway mucosa.[21-27]

Carcinoma of lung:

Like any other malignancy, Lung cancers arise by accumulation of genetic changes which alter and modify the normal bronchial epithelium to malignancy. Usually these tumours found to arise in and around the hilum of lung. About 75% of these tumours arises from first to third order bronchi. Most of the peripherally arising lung cancers are adenocarcinomas.

LUNG CANCER TYPES

Small cell carcinoma:

Of all lung cancers, Small cell carcinoma constitutes about 10-20% of them. Mostly found to arise in male patients and in that more than 85% are in smokers. Small cell carcinoma of lung is an highly aggressive malignant tumour. Most commonly it appears as lesion in the central portion of lung , but it may also seen in peripheral regions. Macroscopilly it appears as greyish white to tan, soft, friable and extensively necrotic. Microscopically it appears as solid pattern, but there are other growth patterns like ribbon and streams, or ductules and tubules, rosettes and pseudo rosettes may also be seen .[28,29] according to **betticher et al** [30]

The neoplastic cells are small round, oval or spindled with scant eosinophilic cytoplasm, finely granular chromatin (salt and pepper chromatin), almost absent or inconspicuous nucleoli and nuclear moulding. [30]

Combined small cell carcinoma :

A tumour with characteristics of small cell variety with additional small components of either squamous cell carcinoma or adenocarcinoma. This type usually appears as hilar or periphral mass lesions or often presents with mediastinal lymphadenopathy and or lobar lung collapse.

Among all lung cancers, Small cell variety is the most aggressive malignant tumour. It would have metastasized widely at diagnosis and it is almost incurable surgically.

Non small cell lung carcinomas

Squamous cell carcinoma:

Most cases of squamous cell carcinoma occurs in men. Among smokers it is the most common malignant lung tumour. This tumours are usually large at presentation arising in the central portions of lung, either from segmental or sub-segmental bronchi.[31,32] But now the incidence of this tumours in peripheral of lung is also increasing. Usually it may occurs as hilar or perihilar mass lesion. In central type lesions lobar or entire lung collapse may occur. It has a special tendency to undergo cavitation, central necrosis,etc .

According to **suprun et al**[33], histologically, these tumours shows keratinization (individual cells or pearl formation) with or without intercellular bridges. The degree of differentiation of tumours influence the presence of these features, being more prominent in well differentiated ones and seen focally in poorly differentiated varieties.

Variants of scc:

Papillary variant:

This variant of squamous cell carcinoma shows exophytic and endo-bronchial growth invasion in most of the cases. But sometimes limited intra-epitheial spread without invasion is seen.[34]

Clear cell variant:

This variant of scc contains most malignant cells featuring classical clear cytoplasm.[35]

Small cell variant:

These are poorly differentiated SCC with small tumour cells which retains the morphological characteristics of NSCLCs but with focal squamous differentiation.[36,37]

Basaloid variant:

This squamous cell carcinoma variant shows peripheral palisading of nuclei which is the prominent finding and it usually presents with very aggressive clinical course. Squamous cell carcinoma have better survival rate than adenocarcinoma.[29]

Adeno carcinoma of lung:

Adenocarcinoma is the most common type of lung tumour seen among women and non smoking men. This type usually tends to be smaller and

peripherally located at presentation. But rarely it may also presents in central location as hilar or peri hilar mass lesions. Cavitation is seen rarely.

Adjacent structures like pleura and chest wall are involved in 15% of cases. Presentation with hilar lymphadenopathy is less common with adenocarcinoma than with other types . Grossly the size varies widely and may appear as solitary or multiple mass lesions.

Based on location ,Six macroscopic patterns are recognised.

1. Peripheral tumour (most commom type),
2. Central or endobronchial tumour.
3. Diffuse or lobar pneumonia like tumour. In this variety the underlying architecture is preserved which is a typical feature of mucinous BAC.
4. Bilateral diffuse lung disease.
5. Diffuse interstitial fibrosis or localised scar.[38]
6. Tumour invades widely along the visceral pleura.[39].

Histologically it may appears as well differentiated tumours with well developed glandular pattern to poorly differentiated solid mass.

Histological subtypes of adenocarcinoma:

1. Mixed Type :

Most common type of adenocarcinoma representing 80% of resected specimens.

2. Acinar Pattern:

It contains tubules and acini composed of columnar or cuboidal cells which may secrete mucin.[29]

3. Papillary Pattern :

In this type secondary and tertiary papillary structures are seen which replaces the underlying lung architecture. Tissue invasion and necrosis may be present. The lining cells may be mucinous and non-mucinous secreting cuboidal to columnar cells. Micropapillary pattern of adenocarcinoma, are usually prognostically unfavourable varieties.[40]

4. Bronchoalveolar pattern :

In this pattern malignant cells will grow along the alveolar structures (this is known as lepidic growth). but without vascular, stromal, or pleural invasion.[29]

5. Solid Pattern:

This variety composed usually of polygonal cell sheets which lacks tubules acini and papillae but mucin is seen in atleast five tumour cells.

Variants of Adenocarcinoma of lung:

Mucinous (or colloid) adenocarcinoma.

Fetal adenocarcinoma.

Clear cell adeno carcinoma.

Signet ring adeno carcinoma.

Large cell carcinoma:

These are undifferentiated carcinoma that lacks the architectural and cytologic features of squamous cell or small cell or glandular pattern. 9% of lung cancer patients are due to large cell carcinoma.[41-43] It usually presents as large peripheral mass. Histologically it consists of sheets of large polygonal cells with characteristic vesicular nuclei, nucleoli and moderate cytoplasm.

Variants :

1. Large cell neuroendocrine carcinoma :

This type constitutes 3% of lung cancers.[42]. The malignant cells are arranged in various patterns such as organoid, nesting, trabecular rossetes or peritubular palisading paterns. [29,44]. The cells are usually large with abundant cytoplasm and nucleus shows prominent nucleoli.

2. Combined large cell neuro endocrine carcinoma :

This tumor shows combination of features of squamous cell carcinoma, adenocarcinoma, giant cell carcinoma and may be spindle cell carcinoma too.

3. Basaloid carcinoma:

Here, the tumor cells are arranged in many patterns as nodular, solid, trabecular, and invasive growth pattern . May be Peripheral palisading of cells are noted. The cells are monomorphic, small cuboidal to fusiform with nuclei showing moderate hyperchromatism. [45]

4. Lymphoepithelioma like carcinoma:

They show growth pattern, with tumor cells having large vesicular nuclei, and prominent nucleoli. This type of carcinoma show heavy lymphatic infiltration .[42-44,46]

5. Clear cell carcinoma:

Large polygonal tumour cells with clear, foamy cytoplasm.[47,48]

6. Large cell with rhabdoid phenotype:

Rhabdoid cells containing tumour in which this rhobdoid cells should constitute atleast 10% of tumour cells.

Adeno squamous carcinoma:

It occupies about 0.4 to 4 % of all lung malignancies. Common at periphery of the lung. Microscopy shows features of both squamous cell carcinoma and adeno carcinoma, in which, each type should contribute atleast 10% of the tumour.[49-51]

Sarcomatoid carcinoma:

It occupies about 0.3 to 1.3% of all lung malignancies. Microscopically this shows components of sarcoma or sarcoma like differentiation. Five subtypes in this are pleomophic type, spindle cell type, giant cell type, carcinosarcoma and pulmonary blastoma type.[52-56]

Carcinoid tumours:

Typical and atypical carcinoids are the major types of carcinoid tumor.

Other types of lung malignant neoplasms:

Other rare types include adenoid cystic carcinoma, mucoepidermoid carcinoma, lymphomas, sarcomas, and epithelial- myoepithethelial carcinoma.

CLINICAL FEATURES:

Symptoms include cough , chest pain, hoarseness , haemoptysis, ,shortness of breath , fatigue, weight loss, new onset of wheeze (common in squamous cell carcinoma)

Adenocarcinomas are usually asymptomatic, and more often a incidental radiologic finding.

Small cell carcinoma is present with symptoms pertaining to distant metastasis.

The local effects of lung tumor can be obstructive pneumonia, pleural effusion, chest pain/back pain which is due to mediastinal invasion by the tumor itself.

Nerve entrapment by the tumor, to say, recurrent laryngeal nerve causing hoarseness, sympathetic nervous system leads to horner syndrome, phrenic nerve leads to diaphragmatic paralysis.

Superior vena cava syndrome due to SVC obstruction by tumor

Pericardial involvement produce pericarditis and cardiac tamponade

Dysphagia due to invasion into oesophagus.

Metastasis leads to:

The symptoms related to the organs involved. Involvement of liver, pancreas and adrenals produce symptoms like loss of weight, abdominal pain. Bone metastasis produce bone pain.

CNS involvement causes neurological symptoms such as dizziness, head ache, and vomiting.

Paraneoplastic syndromes common with lung carcinoma are hypocalcemia, hypercalcemia, carcinoid syndrome , cushing syndrome, gynacomastia etc.

Other systemic manifestations :

Lambert-Eaton myasthenic syndrome due to autoantibodies directed against neuronal calcium channels[57]. It is charecterised by peripheral neuropathy, acanthosis nigricans, hypertrophic pulmonary osteoarthropathy, hematologic abnormalities like anemia, thrombocytopenia, eosinophilia, leukemoid reaction, leukoerythroblastosis, etc.,

Course of the Disease:

Lung carcinomas are usually preceded by dysplasia lasting for years followed by carcinoma in situ for several years and presents as a mass lesion which becomes symptomatic. The lesion appears as a gray white ,firm to hard, which may show haemorrhage and necrosis at some foci.[20,21]

The neoplasm can grow intraluminally and present as a mass within the lumen. The tumour can also penetrate the wall of the bronchus and involve adjacent peribronchial tissues, carina, mediastinum etc.

Local extension to pleura, pericardium, and the adjacent lymph nodes such as tracheal, bronchial, mediastinal lymph nodes may occur.

Distant metastasis occurs via both lympatic and haematogenous routes. All tumours except squamous cell carcinoma will metastasize early . Distant metastasis to adrenals is more common (>50%), followed by liver (30-50%), bone(20%), and brain(20%).[46]

Imaging in Lung Cancers:

Pre and post diagnostic imaging done for various reasons

1. For finding a suspicious area that might be malignant.
2. For staging of the disease.
3. Inorder to assess the effectiveness of treatment
4. For the surveillance for recurrence of tumor

T.V Colby et al[59] found that the various imaging modalities that have their role in diagnosing lung cancer are

X-ray:

The primary investigation to detect and characterise the lung mass. It helps in assessing the involvement main bronchi / trachea. Also , helps in identifying lymphadenopathy , mediastinal invasion, and pleural effusion.

Computed Tomogram:

This helps to locate the tumor. helps in assesing the size and shape of tumours. Also the adjacent areas of involvement and any metastastic lesions in adrenals, liver , brain and other internal organs can be found.

Magnetic Resonance Imaging:

Metastatic lesions of brain and spinal cord etc., can be detected

Ultrasonogram:

It detects pleural effusion. also guides for thoracocentesis, and diagnostic biopsy of peripheral lung and lesions of mediastinum.

Positron Emission Tomography(PET scan):

A form of nuclear imaging which detects biochemical changes in body tissues. Often, it is used as a whole body scan to detect early metastatic lesions and tumour recurrence.

Literature stated that staging of cancer patients has been improved with the use of PET [60-62]

Bone Scan:

To detect any metastatic lesions in bones

INVESTIGATIONS TO DIAGNOSE LUNG CANCER:

The gold standard investigation for the diagnose of lung cancer is tissue diagnosis under microscopy. Many diagnostic techniques are available to obtain tissues are

1. Sputum cytology
2. Thoracentesis
3. Excisional biopsy of accessible nodes
4. Flexible bronchoscopy (FOB) with or without transbronchial needle aspiration
5. Transthoracic needle aspiration
6. Video-assisted thoracoscopy, and
7. Thoracotomy [63].

In view of selecting the appropriate test procedure, the diagnosing physician, should determine which type of lung cancer is suspected.

Thoracotomy can be done in patients suspected to have early stage disease, which appears amenable to surgery. [63-67]. Staging and tissue diagnosis can be done with this [68].

To diagnose with sputum cytology, at least 3 samples of sputum must be collected; since this is a non-invasive test, even if it is negative, further testing can be done.[60,65,68] Presence of hemoptysis warrants sputum cytology. Also,

centrally located tumors needs sputum cytology. Specificity of sputum cytology in diagnosing lung cancer is 99% and its sensitivity for centrally located lung tumors is 71%, and for peripheral tumors is <50%. [60,66,69] It also aids in diagnosing squamous cell carcinoma.

Thoracocentesis can be performed in case of pleural effusion. Malignacies of lung can be diagnosed with pleural fluid sampling. The sensitivity of thoracocentesis in diagnosing lung cancer is 80% and its specificity is < 90%.

In order to get a tissue sample, biopsy of an accessible lymph node may also be taken.

If the stage of the cancer types are not clear, sputum cytology, flexible bronchoscopy (FOB), and transthoracic needle aspiration are the recommended test procedures.

Flexible bronchoscopy is done by passing scope along the bronchial lumen and taking tissue samples by bronchial washings and/or by biopsies. The sensitivity of flexible bronchoscopy in detecting the lung cancer is 88%.

According to **De Wever W et al**[60] Placing catheters into patients lung should never be attempted without the guidance of Computerized tomography (CT). The sensitivity as well as specificity of this test depends on the location

of tumor and site of tissue sampling. The sensitivity in diagnosing the centrally located tumors with the help of flexible bronchoscopy is 88%. The specificity for same is 90%. The sensitivity for peripherally located tumors falls to 60 to 70% with this technique.

According to **Rivera MP et al**, With the guidance of CT or fluoroscopy, transthoracic aspiration with the use of appropriate needle is the procedure of choice recommended for peripherally located tumours with sensitivity of 90% and specificity of 97%. If the transbronchial needle aspiration done in a patient with peripheral tumour is not conclusive, and if the patient is not suitable for surgery, this technique is recommended. A major complication of this procedure is pneumothorax, which is seen in 25 to 30% of the patients undergoing this procedure .

Small peripherally located tumours with size < 2 cms in diameter, pleural effusion, pleural tumours can be proceeded with Video assisted thoracoscopy. Endoscopes are helpful in visualising the space between lungs and parietal pleura. It also helps in detecting the small lesions in inter pleural space, to take tissue biopsy and to resect some lung cancers in early stage. It can prevent the attempt of thoracotomy, which is the major advantage of this procedure.

Lastly in all cases where the tumour is resectable, thoracotomy is routinely recommended for diagnosis and as treatment for early stage disease[60,70].

SCREENING:

Since we know many types of lung cancer histologically, finding a single biomarker is in fact a great challenge. Several such biomarkers are being evaluated. The effective screening programs could help for early detection of lung cancers which may increase the survival. One of the research projects at the Moffitt Cancer Research Center, has such an objective [71,72]. Present research emphasises microscopic examination of sputum sample staining patterns. One of the possible screening tool is monoclonal antibodies (Mabs). The pattern and the intensity of stain of the Mabs and varying cell characteristics are now being analysed. The genetic and protein markers helps in more understanding of tumor biology[72]. The process of epithelial carcinogenesis can be due to mutation of some particular genes, which can alter its control over abnormal cell growth. Heterogeneous nuclear ribonucleoprotein (hnRNP) is found to be useful marker for early detection of the disease in sputum cytology. Datas suggest that hnRNP is being expressed in most of the lung cancers before any morphologic abnormalities detected.

Other important biological markers found in lung cancers include: Tumor suppressor genes such as p53, Rb, p16, p21, the proto-oncogenes such

as c-myc, c-erB-1, K-ras , HER-2, HGF and growth factors such as TGF-b, GRP/BN, PTHrP, IGF-I & II , FDGF, apoptotic factors and factors favouring angiogenesis such as Bcl-2, VEGF, respectively and gene amplification factor, HER-2 [71] These molecular markers are important in diagnosing pulmonary malignancies.It also helps to determine the prognosis as well as treatment regimen. According to the study presented by Duarte,et. al, 2005, many biological markers are found to be associated with greater frequency with various tumors[73] . Rb is found to be associated with 30% of NSCLC, whereas Rb gene is found to be associated with positive in 100% of SCLC.

The present clinical trial conducted by National Cancer Institute on a large scale called as Prostate Lung Colorectal and Ovarian Screening Trial (PLCO)[74,75]. Its main objective is to ascertain the efficacy of screening tools used in trial and to evaluate the mortality rate associated with the specific type of malignancy under study [142]. The main disadvantage of this trial is that the conventional chest x-ray fails to detect the lung malignancies in early stages[74]. Another such study is the the National Lung Screening Trial (NLST). This trial compares spiral CT scans with the conventional chest x-rays and determines which screening tool is more effective in reducing the mortality due to lung cancers. Spiral CT detects the lung nodules which are be detected by conventional chest x-rays; This has created moral and ethical issue

since spiral CT has been proved to detect the lung cancers at early stages as compared to chest x-rays [76].

CLASSIFICATION OF LUNG CANCER

WHO Classification (ANNEXURE II)

IASLC/ATS/ERS Classification in small biopsy and cytology (ANNEXURE III)

Prognostic factors:

Early detection of cancer favours increased survival; but unfortunately, none of the screening programmes are proved to be successful[79]. Due to lack of early detection of this malignancy, it has become one of the most lethal among all cancers; Mortality rate in lung cancer have superceded the same due to colorectal, prostate and breast cancer combined. Patients are asymptomatic till the advanced stage, which is the major drawback. American Cancer Society observed that only 15% of these cancers are diagnosed in early stages , i.e. Stage I. In patients with lung cancers, the five year survival rate is 15%, which is due to the lack of programmes for early diagnosis .

National cancer institute showed that, in lung cancer, the 5 yr survival rate is only 15% for whites and the same for blacks is 11%.[80,81]

Age :

Lung cancers in patients < 40years will have poor prognosis. The aggressiveness of the cancer and presentation at advanced stages are the probable cause for this..[80]

Sex :

Women have worse prognosis than men. This is partially due to the fact that, women have high incidence of lesions in advanced stage and adenocarcinoma is the commenest type in women.[81,82]

Location :

Superior sulcus tumors have better prognosis rather than tumors at other site. Peripherally located squamous cell carcinomas have better prognosis than those located centrally.[83-85]

Stage of the disease :

Tumour size :

Large tumours have worser prognosis than small tumours of same histological type. In adenocarcinoms showing both in-situ and invasive components, the site of the invasive component is an independent predictor of the survival rate.[86-88]

Cell type and degree of differentiation:

- Squamous cell carcinoma is found to be the most type of lung cancer among other lung cancer types.[89-92] For well differentiated tumours, 5 year survival rate is 40% for those undergoing resection, for moderately differentiated tumours, it is 20% and for poorly differentiated tumours 5 year survival rate is 7% .
- Among adenocarcinomas, papillary carcinoma showing micropapillary pattern have the worst prognosis than other types. Prognosis of bronchio alveolar carcinoma seems better than ordinary adenocarcinoma.[93-94]
- The presence of tumour giant cells in large cell carcinoma have worse prognosis.[95]
- Small cell carcinomas have worse prognosis. The 5 year survival rate for small cell carcinoma patients is only < 2%[95]
- If the lymphoplasmacytic component is found prominently in tumor under microscopy, then it favours good prognosis.[94]
- Stronger expression of TTF -1 in patients with NSCLC, indicates better survival.[96,97]

Other poor prognostic factors are :

- Local and regional extension of the tumor, to say, chest wall invasion, vascular invasion, regional lymph node involvement .[98]
- The presence of tumor cells in plueral fluid [99].

- Peripherally located adenocarcinomas and also undifferentiated large cell carcinomas which are found to be in association with fibrotic scar.[100,101]
- The presence of rhabdoid cells.[78]
- RAS and P21 expression in NSCLC, and NMYC gene expression in small cell carcinomas[102-105]
- p53 and HER 2/neu over expression [102-105]

TREATMENT FOR LUNG CANCER

Four basic modes of treatment available for lung cancer

1. Sugery
2. Radiation therapy
3. Chemotherapy
4. Targeted therapy

Treatment plan varies with many factors such as the type of cancer, stage at presentation, side effects of particular treatment, patients preference, health of the patient.

If NSCLC is confined to the lung, it is considered as early stage of the disease and surgical resection can be done.[106] Post operative radiotherapy with or without chemotherapy can be considered.[107]. If patient cannot withstand surgery, radiotherapy is the choice of treatment.

Various types of surgeries performed are lobectomy, wedge resection, segmentectomy and pneumonectomy.

Early stage samll cell lung carcinoma should be treated with chemotherapy and concurrent radiotherapy.

Once the tumor started spreading beyond hemithorax with metastasis to mediastinal lymph nodes, surgery is no longer recommended and the treatment of choice includes radiotherapy and chemotherapy.

External beam radiotherapy and radioisotope therapy are the two types of radiotherapies given in lung cancers.

Chemotherapy includes platinum based Cisplatin and Carboplatin([107]) and non platinum based drugs such as Docetaxel, Paclitaxel, Gemcitabine, Irinote can.

Distant metastasis is treated with chemotherapy alone.([107])

MOLECULAR TESTING AND TARGETED THERAPY

Molecular testing reveals various specific charecters of the tumor which influences the diagnosis, prognosis and treatment with targeted therapy.

Targeted therapy have promising benefits if given to appropriate patients. Here, the therapy is targeted against cancer specific genes / proteins.

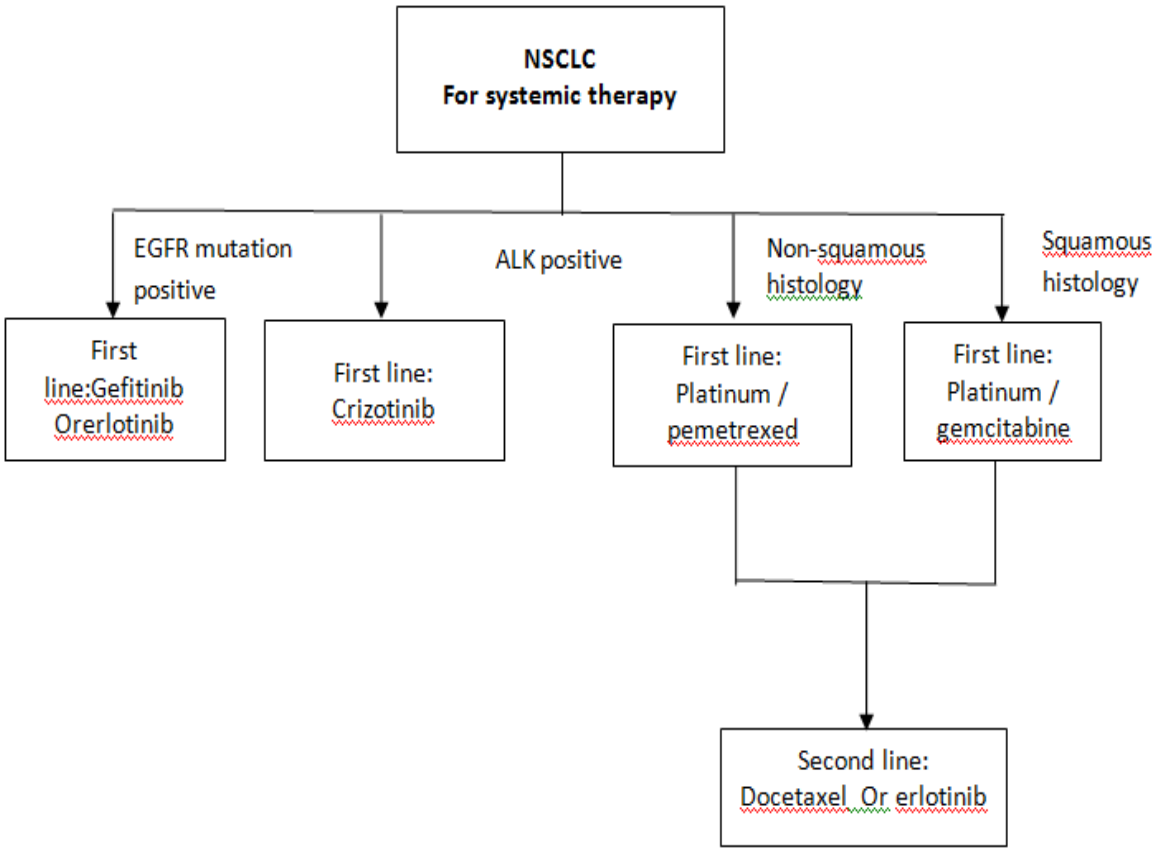
Anti angiogenesis therapy blocks the formation of new blood vessles. Bevacizumab, a monoclonal antibody targeted against VEGF is a potential inhibitor of angiogenesis.

EGFR mutations are seen in 10% - 15% of NSCLC, and common in adenocarcinomas. Geftinib and Erlotinib are the tyrosine kinase inhibitors targeted against intracellular tyrosine kinase domain of EGFR. Hence, tyrosine kinase inhibitors are the first line treatment for NSCLC.[108]

Rearrangement of ALK gene is seen in 5% of NSCLC and is most common in nonsmokers with adenocarcinoma. Drugs that are targeted against ALK mutation includes, Crizotinib and Certinib.[109-111]

Several new markers are evolved which may be associated with the outcome on targeted therapy. They are ROS1, BRAF , FGFR, HER2, PIK3CA, KRAS.

Treatment for NSCLCs in advanced stage:



RECOMMENDATIONS BY IASLC/ATS/ERS NEW MULTIDISCIPLINARY INTERNATIONAL CLASSIFICATION, FOR SMALL BIOPSY AND CYTOLOGY SPECIMENS WERE:

- For cytology as well as small biopsy specimens, if a clear differentiation can be done, which satisfies the standard morphologic criteria, further specific typing of NSCLC into squamous cell carcinomas and adenocarcinomas can be done with morphology alone.

- The term NSCLC - NOS must be used as infrequently as possible and it should only be used if the diagnosis cannot be made out by morphology and /or by special staining / IHC.

- When small biopsy / cytology specimen is used in addition with special stains for diagnosis, it should be clearly noted whether the diagnosis is achieved with only light microscopy or in combination with special stains.

- The term non-squamous cell carcinoma which is used by clinicians, should not be used by pathologists while reporting. Pathologists should report NSCLC only as ADC , SQCC and NSCLC - NOS.

- The tissue specimens received by pathologists just be used judiciously and preserved to the maximum, as more tissues will be needed for further molecular studies.[113-115]

- In small biopsies / cytology specimens, if any invasive pattern is found in adenocarcinoma ,it is to be reported as a lepidic growth pattern . The term minimally invasive ADC and ADC- in situ should not be used.

- The term large cell carcinoma, should be used only in resected specimens as thorough sampling of tumour is not possible in small biopsy/cytology specimens.

- If the tumor shows sarcomatoid features characterised by malignant giant cells or spindle cells with nucleus showing pleomorphism should be classified according to guidelines above as NSCLC favouring ADC or NSCLC favouring SCC based on features of glandular pattern or squamous features respectively. when these features are absent it is to be reported as NSCLC - NOS with a word about sarcomatoid features..

- Only if the tumor shows neuro endocrine morphology, neuro endocrine IHC markers are performed then.

- Further classification of NSCLC- NOS is possible with the use of IHC, into NSCLC favouring ADC and NSCLC favouring SCC.

- It is advised to use minimal stains for further subclassification of NSCLC-NOS.

- It is recommended to use only one marker for adenocarcinoma or one marker for squamous cell carcinoma.

- Currently, the single best marker for diagnosing adenocarcinoma is TTF-1. Staining with diastase - periodic acid schiff, alcian blue/ PAS stains or mucicarmine also play a role in diagnosing adenocarcinoma.

- The specific marker for diagnosing SCC is Polyclonal p40 rather than the monoclonal p63 . p40 is likely to surpasses p63 as a best IHC marker in diagnosing squamous cell carcinoma.

- In NSCLC -NOS , the cases which shows **TTF-1 positive and /or mucin positive**, but p40 and p63 negative are termed as **NSCLC favouring adenocarcinoma**. similarly those cases with **p40 and/or p63 positive** but TTF-1 and mucin stain negative are termed as **NSCLC favouring SCC** with comment on whether special stains are used to arrive at diagnosis.

- In case, one population of tumour cells show TTF-1 reactivity and another population of tumor cells show postive for squamous cell markers, possibility of **adenosquamous carcinoma** should be considered.

- But if TTF-1 as well as p40 are negative and fails to show any squamous or glandular morphology, the diagnosis still remains as **NSCLC-NOS.**

ALTERATIONS SUGGESTED BY IASLC/ERS/ATS INTERNATIONAL CLASSIFICATION OF LUNG MALIGNANCY IN RESECTED SPECIMENS:

1. The term bronchoalveolar carcinoma is discarded.

✓ In the new multidisciplinary classification, BAC is discarded. Originally, broncho alveolar carcinoma is defined as a non-invasive lesion, but since then, it is used to denote broad group of tumours which includes

- Nonmucinous BAC. This is defined as solitary non invasive small peripheral adenocarcinoma. This type will have 100% 5 year survival rate.[116]
- Minimally invasive small peripheral adenocarcinoma with 5 year survival upto 100%.[117,118]
- Invasive adenocarcinoma with mixed subtype.[119,120]
- Nonmucinous and mucinous adenocarcinoma, which is known as BAC earlier.[120]
- Advanced mucinous adenocarcinoma(stage 4) with low survival rate.[4,6]

✓ In the new multidisciplinary classification, 'BAC' is referred to as "former BAC"

2. New concepts were introduced for

- ✓ Small solitary peripheral adenocarcinoma with size less than or equal to 3cm,with pure lepidic growth without invasion with 100% disease specific survival **as adenocarcinoma in situ(AIS).[116]**

- ✓ Small, solitary peripheral adenocarcinoma with size less than or equal to 3cm, with predominantly lepidic growth with invasion, with 100% disease specific interval **as minimally invasive adenocarcinoma(MIA).[117,118]**

3. Former invasive adenocarcinoma with mixed subtype is replaced by predominant pattern..

- ✓ According to 2004 WHO classification more than 90% of lung adenocarcinoma are of mixed subtypes. In this new international classification this mixed subtype is replaced with predominant pattern. It is recommended to choose one predominant pattern based on recording of patterns in 5% increments.[121,122]

4. In multiple lung adenocarcinoma patients it is recommended comprehensive histological subtyping of heterogenous, complex lung adenocarcinoma to determine if the tumours are synchronous, metachronous or metastasis.[123]

5. It recommends the term lepidic predominant adenocarcinoma in place of previously classified as mixed subtype ,predominantly non mucinous BAC.[117]

6. New histological type of Micropapillary predominant adenocarcinoma is introduced . This is associated with poor prognosis.[122]

7. In new international classification, invasive mucinous adenocarcinoma, fetal, enteric and colloid adenocarcinomas are introduced as new variants.[124]

Acccording to **Edwards et al [127],** only 10-15% of lung cancer patients undergo resection and the preoperative diagnosis confirmed. So treament for most of the patients is based on diagnosis with small biopsy/cytology specimens alone.

According to **Suprun et al**, [33]

- The criteria for diagnosis of SCC are

  The presence of Keratin formation and/or Intercellular bridges. In cases if the tumor lacks such features, the intraepithelial in- situ like extensions along the bronchus are present in SCC. Both adeno and small cell carcinoma do not replace the bronchial epithelium to a considerable extent. And most of these cases are either well differentiated or moderately differentiated. This feature aids in histological typing of lung cancers in small biopsy specimens.[125]

  The grading of squamous cell carcinoma cannot be done in small biopsy specimens.[126]

- The diagnosis of adenocarcinoma seems to be more challengeable as the presence of mucin and gland formation are frequently not present in small biopsies, which calls for mucin stains to demonstrate the presence of glandular elements.

- According to the study of **Edwards et al,**[127] the diagnosis of large cell carcinoma is possible only with resected specimens and not on small biopsies. This is because, they are the poorly differentiated forms of adenocarcinoma, squamous cell carcinoma, or neuroendocrine carcinoma and also most major types of lung carncers contain foci of features of large cell carcinoma .

- Recent data showed that the high percentage (30-50%) of NSCLC-NOS has been diagnosed in small biopsies.[128-130] and data from the registry of epidemiology surveillence shows the increasing frequency of this diagnosis.[131]

ALCIAN BLUE/PAS (Mucin stain) :

Mucins are a glycoprotein with high molecular weight, that was synthesized and secreted by epithelial mucosal cells, mainly the goblet cells [132]. Mucins are classified into acidic mucins and neutral mucins histochemically. This will include sialomucins and sulphamucins.

Many adenocarcinomas like cancers of colon, breast, ovary, lung, etc will increase mucin production[133].

Acid mucins and neutral mucins will be differentiated using Alcian blue- PAS staining.[134] This property will be of value in demonstrating even minimal acid mucin. Mucin stains is a valuable markers but its use in lung carcinoma in terms of sensitivity and specificity may be variable. This is a defining characteristics of lung adenocarcinoma, although not positive in all cases.[134]

THYROID TRANSCRIPTION FACTOR-1:

TTF-1 is a DNA binding protein expressed normally in thyroid, lungs, and also in specific locations of diencephalon[135]. Its role in lung tissues is, it regulates the gene expression of surfactant and clara cell secretory protein. the study of such transcription factors in human lung tumours are helpful in understanding the molecular events of the noeplastic transformation of such lung tumors [136].Several reports says that in lung cancers the expression of TTF-1 varies with different histological subtypes[137-140].

According to **J.Korean et al**[143], TTF-1 was expressed in 62.5 - 90% of ADC and in 89-100% of small cell carcinomas, but it was not found or very less frequently found in SCC (0-25%) and large cell carcinomas (0-24%). Many reports highlights the usefulness of TTF1 in differentiating lung cancer

from non pulmonary tumours.TTF-1 appears to be more sensitive and specific marker for diagnosing adenocarcinomas and small cell carcinoma of lung. It is a useful prognostic marker and used in conjucation with proliferative marker ki 67.[143]

p40(p63 delta):

p40 (p63 delta), a new marker, is the shortest variant of human p53. It is a valuable marker in cases where p63 has been traditionally used. p40 has equal sensitivity as p63 in detecting squamous cell carcinoma.

But p40 exhibits superior specificity when compared with p63. Moreover, p40 helps in the early detection of breast cancers , prostate cancers and lung cancers.

In lung cancer, the p40 is a very specific marker for squamous basal cells. It helps to differentiate squamous cell carcinoma from ADC. In breast cancers, the p40 stains the myoepithelial cells along the ducts of mammary glands, and helps in detecting ADH and DCIS.

In prostate cancer, p40 staining of basal cells inside the ducts of the prostatic glands will indicates PIN.

p40 and p63 have equal sensitivity in pulmonary squamous cell carcinomas, but former is more specific. In rare instants, p40 is positive for adenocarcinomas.but it will be weak, focal, and seen in isolated malignant

cells, easily distinguisable from squamous cell carcinoma which is diffusely reactive.

p40, a nuclear marker has excellent sensitivity and specificity, makes itself a more specific marker for SCC. Diffuse labelling of p40 indicates SCC, whereas its absence warrants a strong discussion against this diagnosis.[144-148]

Materials and Methods

MATERIALS AND METHODS

This study is a retrospective and prospective study of lung cancer in small biopsies conducted in the Institute of pathology,Madras Medical College and Rajiv Gandhi Government General Hospital, Chennai during the period between July 2013 to June 2014.

A total of 13042 cases were submitted to our department the Institute of pathology, Madras medical college during the period of July 2013 to June 2014 for histopathological examination. Out of them, 392 were lung cases. Among them 19 cases were pneumonectomy specimens, 50 were lobectomy specimens and 323 were small biopsies (Transbronchial, Endobronchial, Open, Ultrasound guided biopsies and Computed tomography guided core biopsies).

Inclusion criteria:

FOB biopsies and CT Guided biopsies from clinically and radiologically suspected cases of lung cancer.

Exclusion criteria:

1) Cases treated with prior Chemotherapy and radiotherapy.
2) Small cell carcinoma.
3) Resected specimens.

METHOD OF DATA COLLECTION:

Detailed history of the cases regarding age, sex, site, tumor location, radiological findings, FOB findings, cytological findings were obtained for all the 323 cases. Out of these

Inadequate for opinion	-	23
Non neoplastic	-	95
Suspicious of malignancy	-	12
Malignancy	-	189

Among the malignant cases,

NSCLCs	-	153 cases
Small cell lung carcinoma	-	13
Others	-	23

These 153 Non small cell lung carcinoma cases were reviewed and sub classified based on H&E morphology according to WHO classification criteria. Tumours were sub typed as adenocarcinoma if it showed features of gland formation and/ or mucin production, squamous cell carcinoma if it showed features of keratinazation or intercellular bridges , large cell carcinoma (undifferentiated non small cell carcinoma) if it lacked both glandular or squamous patterns. In these 153 cases, 2 cases were excluded

because they were resected specimens. Out of these 151 cases, based on morphology seen in biopsy further segregation done as follows:

Group 1: Cases that could be subtyped by morphology alone as adenocarcinoma or squamous cell carcinoma (121 cases),

Group 2: Cases that are poorly differentiated non small cell lung carcinoma (NSCLC-NOS) (30 cases).

All 30 cases of poorly differentiated non small cell lung carcinomas (NSCLC-NOS) from group 2 and 10 cases each of poorly differentiated adenocarcinoma and squamous cell carcinoma selected randomly from group 1 were included for special staining and IHC study for further classify the NSCLC-NOS group into favouring adeno or squamous subtypes and to study the efficency of special stains(ALCIAN BLUE/PAS) and markers (TTF-1, p40).

Five –micron thick paraffin sections were cut and stained with combined mucin stain, TTF-1 and p40 and the tumours were sub-typed based on the algorithm followed by the IASLC/ATS/ERS 2011

Alcian Blue / PAS staining was done as per protocol given in ANNEXURE-V.

IMMUNOHISTOCHEMICAL EVALUATION:

Antigen	Vendor	Species	Dilution	Positive control
TTF- 1	BIOGENIX	Mouse	Ready to use	lung Adenocarcinoma
P40	BIOGENIX	Mouse	Ready to use	Lung squamous cell carcinoma

Immunohistochemistry was done as per protocol given in ANNEXURE-VI.

INTERPRETATION AND SCORING SYSTEM:

The immunohistochemically stained slides were analyzed for the presence of reaction, cellular localization(nuclear), percentage of cells stained and intensity of reaction.

In this study, for evaluation of TTF1 and p40 proteins, greater than 10% expression of the tumour marker within tumour cells is considered as positive. Cases with no focal areas of positive staining and with less than 10% staining are considered negative.

STATISTICAL ANALYSIS:

- Immunohistochemical analysis was done in paraffin embedded tisse samples using the statistical package for social science software version 15.5 which consisted computing the frequency counts and percentages for qualitative variables and mean for quantitative variables.
- P value of 0.005 was taken as cut-off point to determine statistically significant results.

Observation and Results

OBSERVATION AND RESULTS

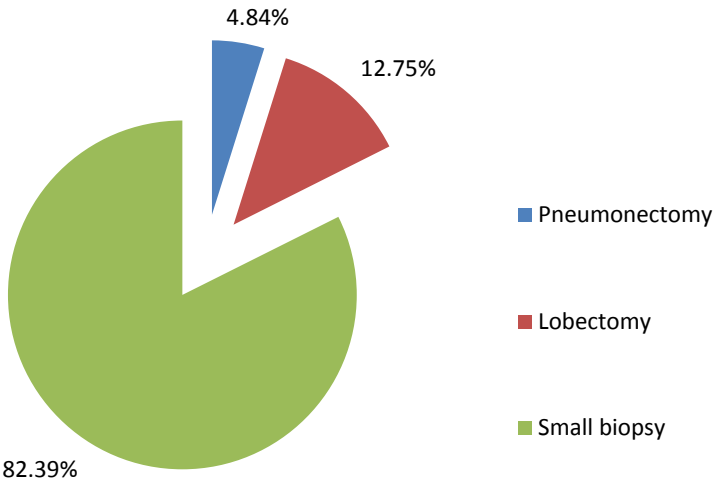
In the study period of 12 months from July 2013 to June 2014,a total of 13042 specimens were received in the Institute of Pathology, Madras Medical College for histopathological examination.

Total numbers of lung specimens received were 392 cases, of these 19 cases were pneumonectomy specimens,50 cases were lobectomy specimens,323 were small biopsy specimens. (TABLE:1 CHART:1)

TABLE 1 : TYPES OF LUNG SPECIMEN:

Pneumonectomy	19(4.84%)
Lobectomy	50(12.75%)
Small biopsy	323(82.39%)
Total	392

CHART:1 TYPES OF LUNG SPECIMEN

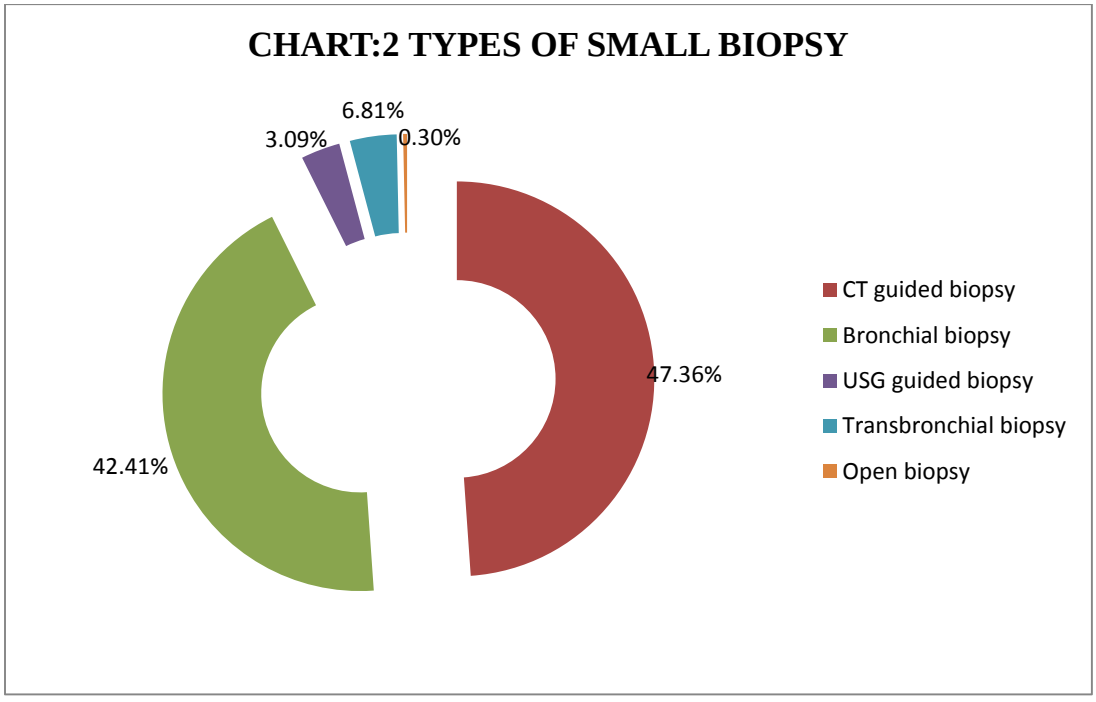


Among the 323 small biopsy specimens ,number of bronchial biopsy were 137,CT guided biopsy were 153,USG guided biopsy were10,open biopsy was 1 and transbronchial biopsy were 22. (TABLE:2,CHART:2)

TABLE :2 TYPES OF SMALL BIOPSY

CT guided biopsy	153(47.36%)
Bronchial biopsy	137(42.41%)
USG guided biopsy	10(3.09%)
Transbronchial biopsy	22(6.81%)
Open biopsy	1(0.30%)
Total	323

CHART:2 TYPES OF SMALL BIOPSY



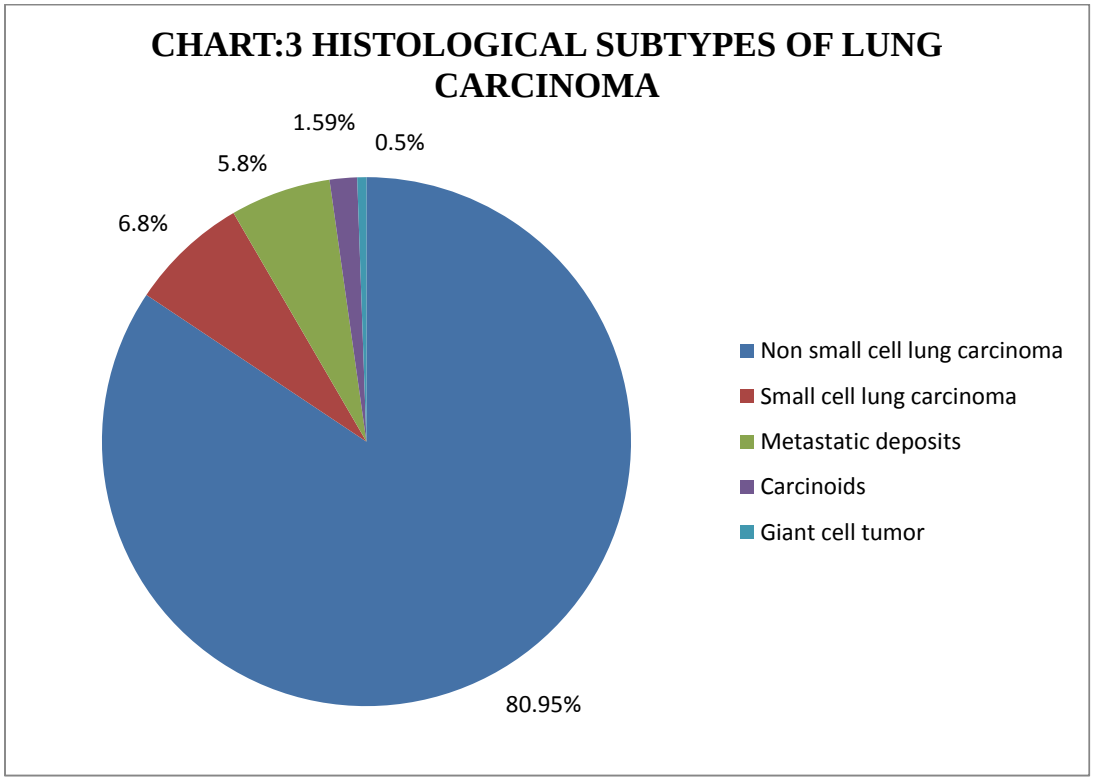
Totally 189 malignant cases have been reported with the percentage of 56.6%, among which 183 malignancies were found to be in small biopsy cases. Among the 183 small biopsy cases 152 were non small cell carcinoma of lung, 13 were small cell lung carcinoma, 2 were malignant mesothelioma , 11 were metastatic deposits in lung, 1 was solitary fibrous tumour, 1 was carcinoid tumor. Out of 6 resected specimen, 1 was giant cell tumor, 1 was large cell lung carcinoma, 1 was spindle cell carcinoma, 1 was SCC, 2 were typical carcinoid tumors.

The most common lung malignancy were NSCLCs (Adenocarcinoma, SCC, adenosquamous cell carcinoma, spindle cell carcinoma, large cell carcinoma) which constitutes 153 cases (80.95%),small cell lung carcinoma constituted 13 cases which accounts for 6.8%.

Metastatic carcinoma constitutes 11cases which accounting 5.8%,the other histological subtypes accounts for 2% .(carcinoids-1%,giant cell tumor-0.5%) (TABLE :3 CHART:3)

TABLE 3:HISTOLOGICAL SUBTYPES OF LUNG CARCINOMA

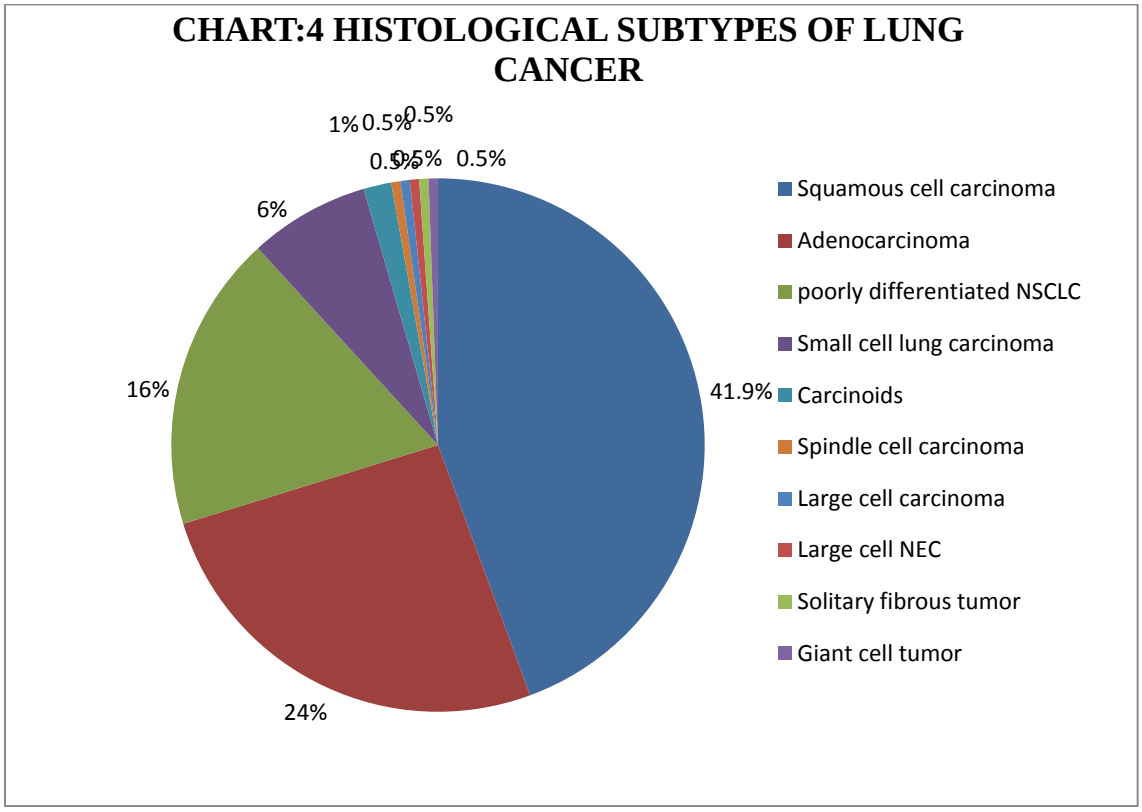
HISTOLOGICAL SUBTYPES	NUMBER OF CASES	PERCENTAGE
NSCLC	153	80.95%
Smallcell lung carcinoma	13	6.8%
Metastatic deposits	11	5.8%
Carcinoids	3	1.59%
Giant cell tumor	1	0.5%



Of the total 189 lung tumours, Squamous cell carcinomas were most common accounting for 79 cases which accounts for 41.9%,adenocarcarcinoma constituted 46 cases accounting for 24%,30 cases were poorly differentiated non small cell lung carcinoma(16%) ,small cell lung carcinoma were 13 cases constituted 6%,11 were metastatic deposits(4%),carcinoids were 3 cases(1%), giant cell carcinoma, spindle cell carcinoma, solitary fibrous tumor and large cell carcinoma each constitutes 1 case and accounting for o.5%. (TABLE: 4& CHART:4)

TABLE:4 HISTOLOGICAL SUBTYPE OF LUNG TUMOURS:

Histological subtypes	Number of cases	Percentage
Squamous cell carcinoma	79	41.9%
Adenocarcinoma	46	24%
NSCLC-NOS	31	16%
Small cell lung carcinoma	13	6%
Carcinoids	3	1%
Spindle cell carcinoma	1	0.5%
Large cell carcinoma	1	0.5%
Large cell NEC	1	0.5%
Solitary fibrous tumor	1	0.5%
Giant cell tumor	1	0.5%

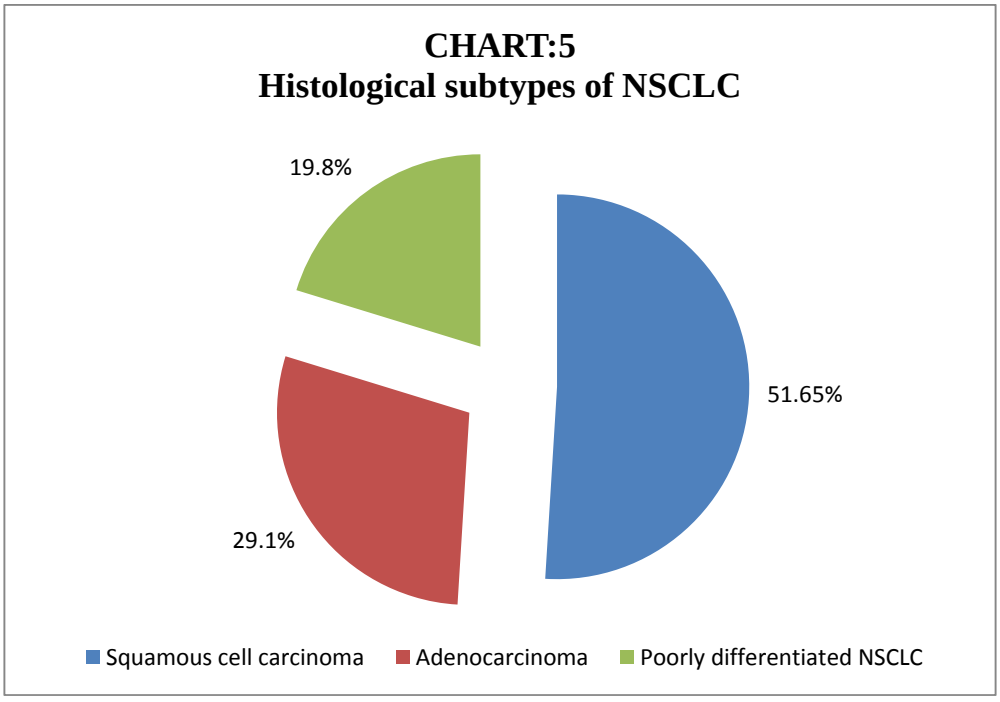


In small biopsy specimen the non small cell carcinoma of lung (NSCLCs) was classified according to WHO classification into squamous cell carcinoma, adenocarcinoma, poorly differentiated non small cell lung carcinoma (which lack squamous or adeno differentiation). Among this SCC of lung were the most common type which accounts for 78 cases (51.65%), adenocarcinoma constituted 44 cases (29.1%), poorly differentiated NSCLC were 30 cases(19.8%), large cell carcinoma was 1 case (0.65%) and large cell neuroendocrine carcinoma was 1 case (0.65%) .large cell neuroendocrine carcinoma was already confirmed by neuro endocrine marker such as NSE and

chromogranin . For Large cell carcinoma there was no material in the block. so these 2 cases were not included in the further study.(TABLE:5 CHART:5)

TABLE 5:NON SMALL CELL LUNG CARCINOMA SUBTYPES ACCORDING TO WHO CLASSIFICATION

Histological types of NSCLC	Numbers of cases	Percentage
Squamous cell carcinoma	78	51.65%
Adenocarcinoma	44	29.1%
Poorly differentiated NSCLC	30	19.8%

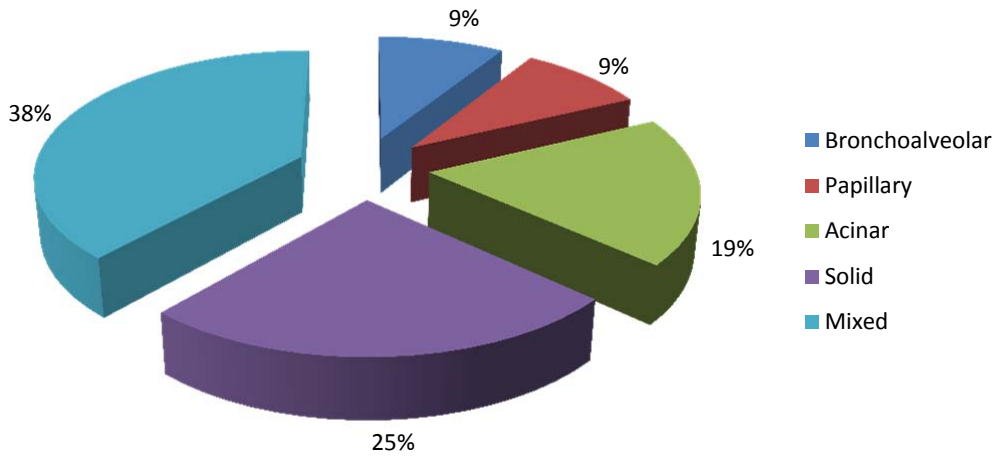


According to WHO classification the adenocarcinomas were subtyped into acinar, papillary, bronchoalveolar, solid, mixed type and grading bronchoalveolar as well differentiated, acinar and papillary as moderately differentiated and solid as poorly differentiated. Among these the mixed type were 17 cases(38.6%), solid type were 11 cases(25%), acinar type were 8 cases(18.8%), papillary type and bronchoalveolar type were 4 each accounts for 9%. (TABLE :6,CHART: 6)

TABLE 6: **ADENOCARCINOMA SUBTYPES ACCORDING TO WHO CLASSIFICATION BY MORPHOLOGICAL FEATURES**

Adenocarcinoma subtypes	Number of cases	Percentage
Bronchoalveolar	4	9%
Papillary	4	9%
Acinar	8	18.8%
Solid	11	25%
Mixed	17	38.6%

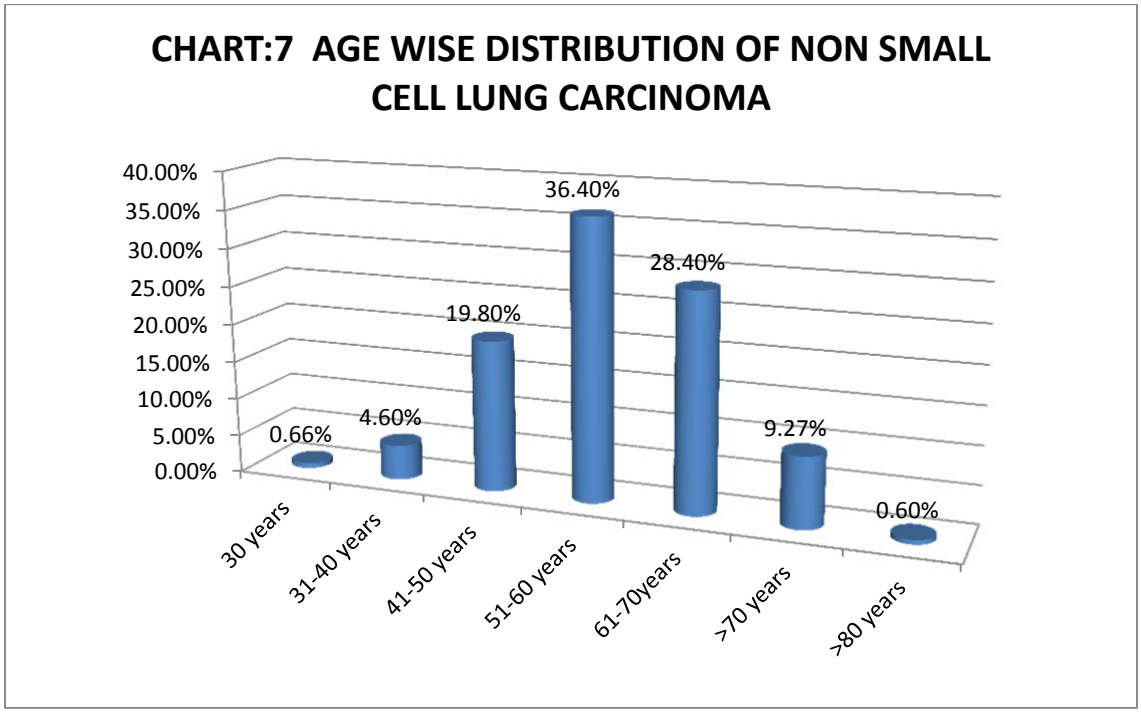
TABLE:6 ADENOCARCINOMA SUBTYPES ACCORDING TO WHO CLASSIFICATION



Peak incidence for all types of non small cell lung carcinoma is 51-60yrs. In this study, the youngest age at presentation is 30yrs. The mean age is 57.62 yrs. 15 cases were observed in the age of above 70 years. 84.76% of the cases were seen in the age group of 40 to 70 years**.** (TABLE:7 & CHART:7)

TABLE 7: AGE WISE DISTRIBUTION OF NSCLCs

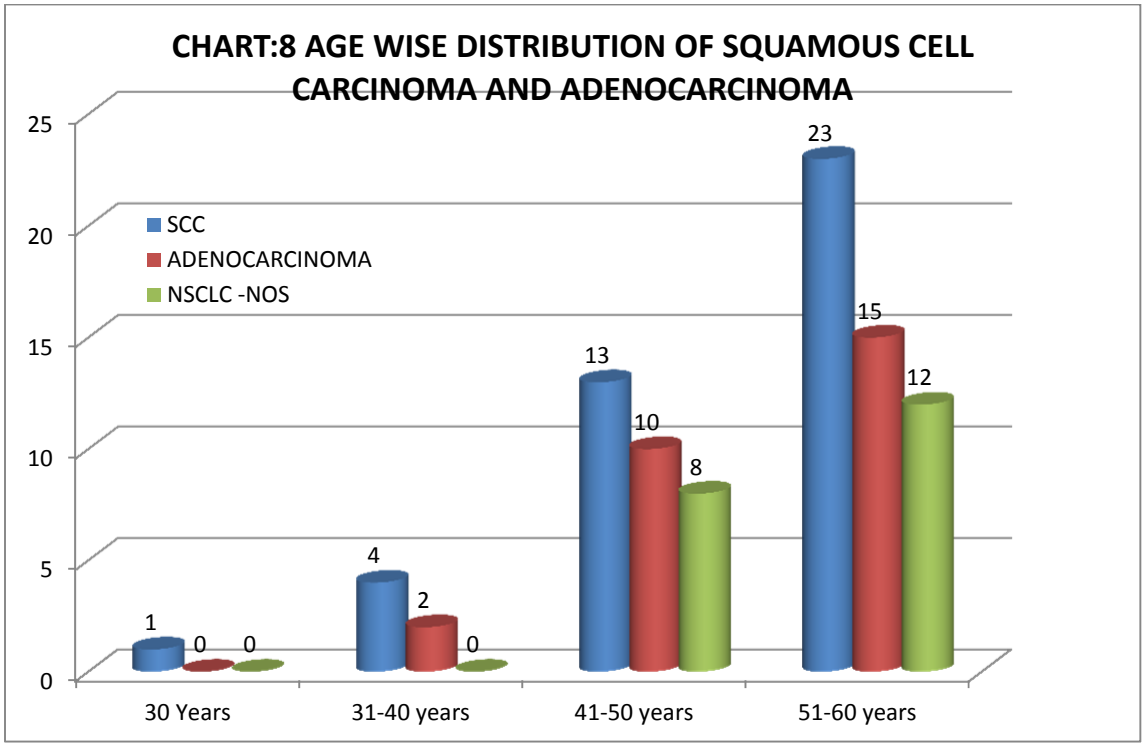
AGE GROUP	Number of cases	Percentage
30 years	1	0.66%
31-40 years	7	4.6%
41-50 years	30	19.8%
51-60 years	55	36.4%
61-70 years	43	28.4%
70-80 years	14	9.27%
>80 years	1	0.6%
Total	151	



Squamous cell carcinoma and adenocarcinoma had a peak incidence in the age group of 51-60 years with mean age for SCC being 57.96 years and for adenocarcinoma 57.02 years. TABLE:8,CHART:8

TABLE :8 AGE WISE DISTRIBUTION OF SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA

AGE GROUPS	SCC	ADENOCARCINOMA	NSCLC - NOS
30 Years	1(1.2%)	0	0
31-40 years	4(5.1%)	2(4.5%)	0
41-50 years	13(16.6%)	10(22.2%)	8(25.36%)
51-60 years	23(29.48%)	15(34%)	12(38.7%)
61-70 years	20(25.64%)	9(20.45%)	9(29.3%)
71 -80 years	6(7.69%)	6(13.6%)	2(6.4%)
> 80 years	1(1.2%)	0	0
TOTAL	78	44	



Among the 78 squamous cell carcinoma, 65 cases (84.4%) were reported in males and 12 cases (15.5%) were reported in females. The male to female ratio was 5.6:1. In adenocarcinoma, males constituted 26 cases (59%) and females constituted 18 cases (40.9%) and the male to female ratio was 1.44:1.

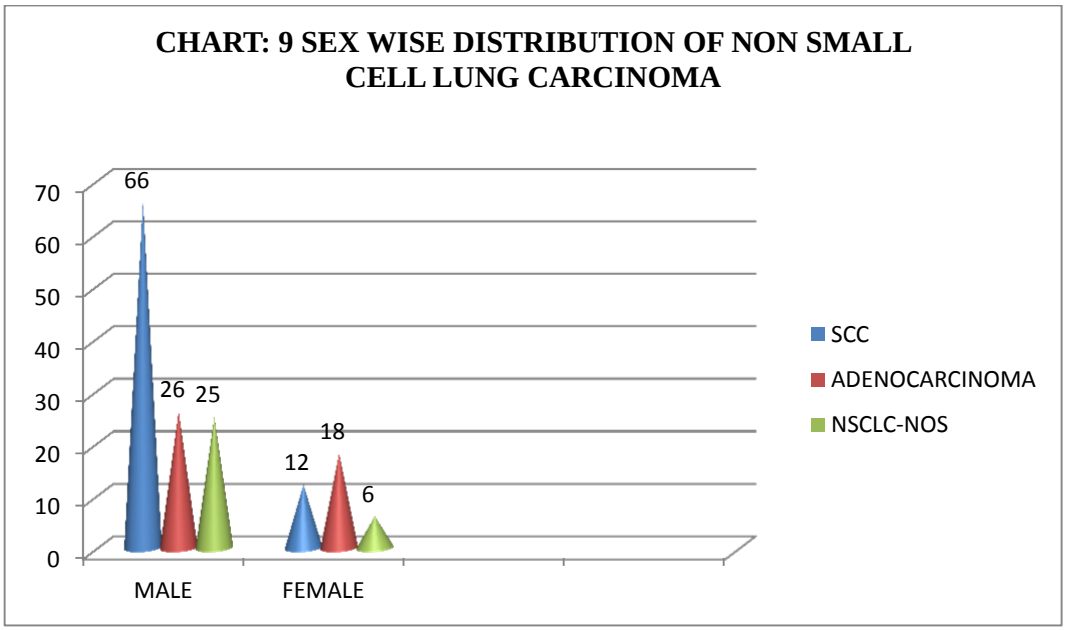
From this study it was observed that both squamous cell carcinoma and adenocarcinoma ,are more common in males than females. But when compared to squamous cell carcinoma ,the females are more frequently affected by adenocarcinoma with near equal incidence in males. (TABLE:9,CHART:9)

TABLE:9 SEX WISE DISTRIBUTION OF N

ON SMALL CELL LUNG CARCINOMA

SEX	SCC	ADENOCARCINOMA	NSCLC-NOS
MALE	66(84.4%)	26(59%)	25(80.6%)
FEMALE	12(15.5%)	18(40.9%)	6(19.35%)
TOTAL	78	44	31

p value-0.005,chi square-10.6



Cough , breathlessness and weight loss were the most common clinical presentation for both squamous cell carcinoma and adenocarcinoma. TABLE:10,CHART:10

TABLE:10 CLINICAL FEATURES OF SQUAMOUS CELL CARCINOMA

Clinical features	Number of cases	Percentage
Cough	71	91%
Hemoptysis	51	65.38%
Breathlessness	21	26.92%
Chest pain	34	43.58%
Weight loss	64	82%

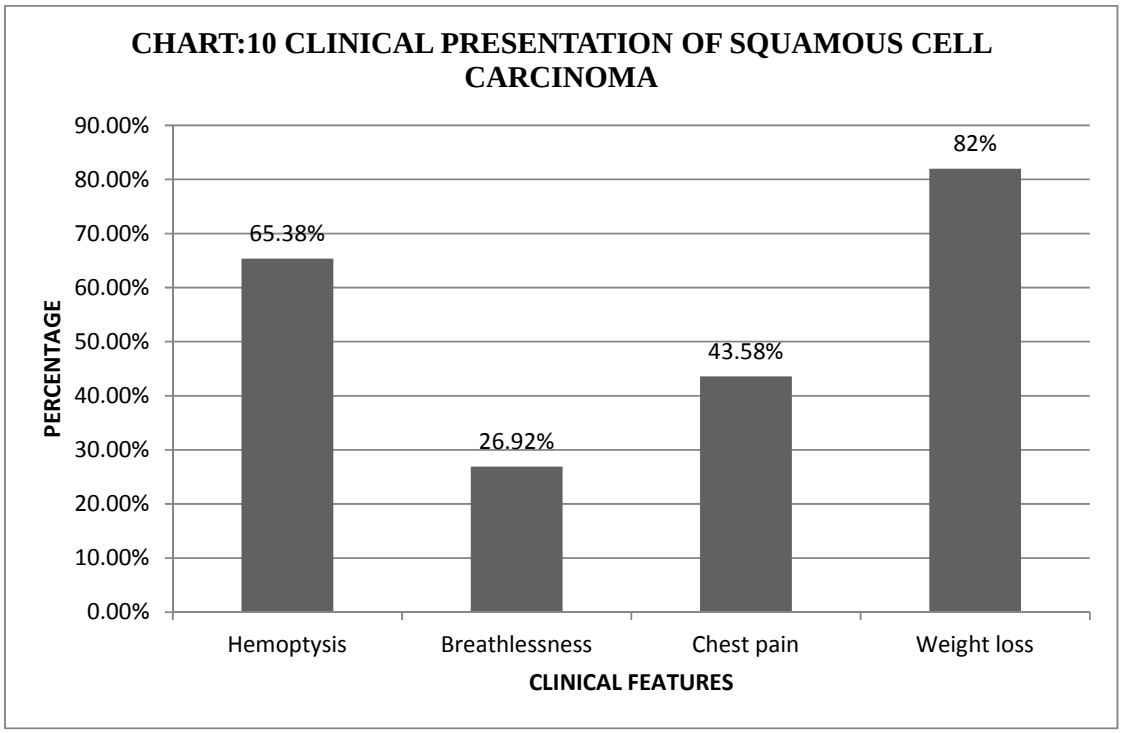
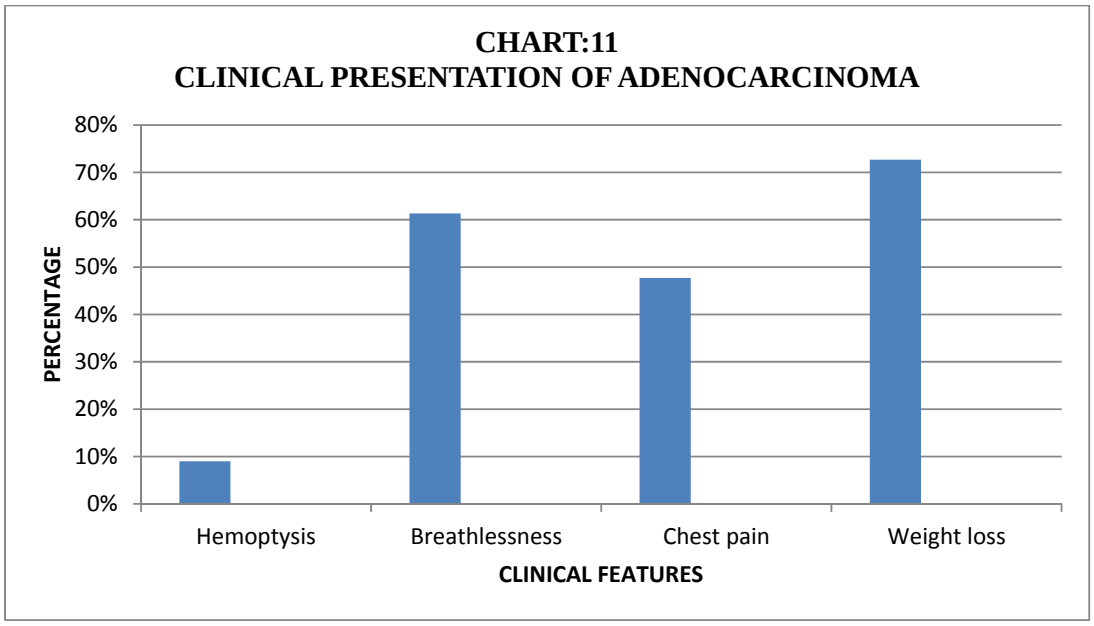


TABLE:11 CLINICAL PRESENTATION OF ADENOCARCINOMA

Clinical features	Number of cases	Percentage
Cough	42	95.5%
Hemoptysis	4	9%
Breathlessness	27	61.3%
Chest pain	21	47.72%
Weight loss	32	72.7%

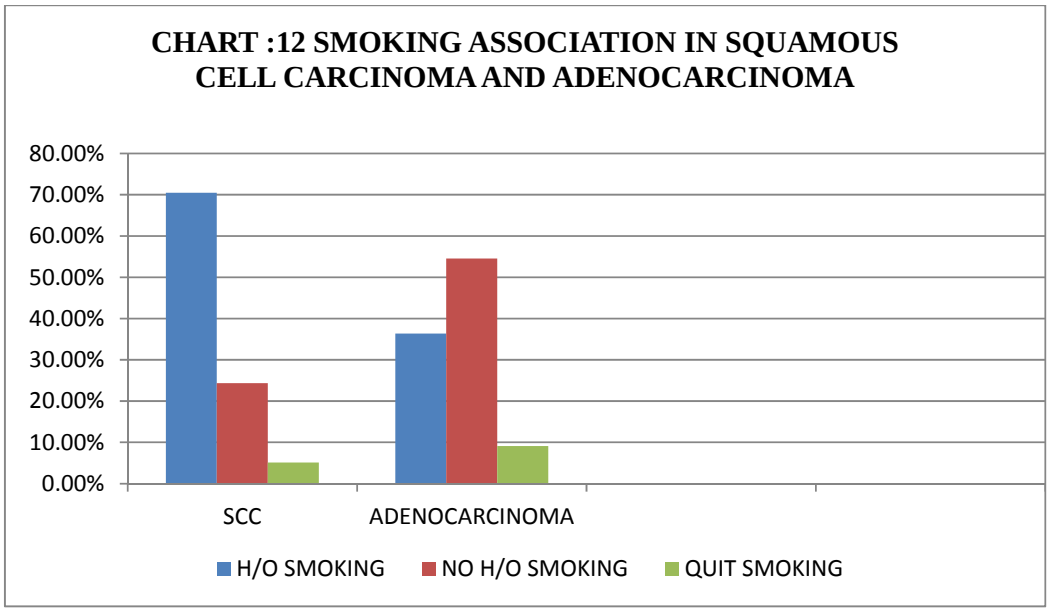


In this study ,squamous cell carcinoma most commonly occured in smokers than nonsmokers and adenocarcinoma were more common in non smokers than smokers.p value-0.001 chisquare test-13.6 TABLE:12, CHART:12.

TABLE:12 SMOKING ASSOCIATION IN SQUAMOUS CELL CARCINOMA AND ADENOCARCINOMA

	H/O SMOKING	NO H/O SMOKING	QUIT SMOKING	TOTAL
SCC	55(70.5%)	19(24.35%)	4(5.12%)	78
ADENOCARCINOMA	16(36.36%)	24(54.54%)	4(9.09%)	44
TOTAL	71	43	8	

p value-0.001 chisquare test-13.6

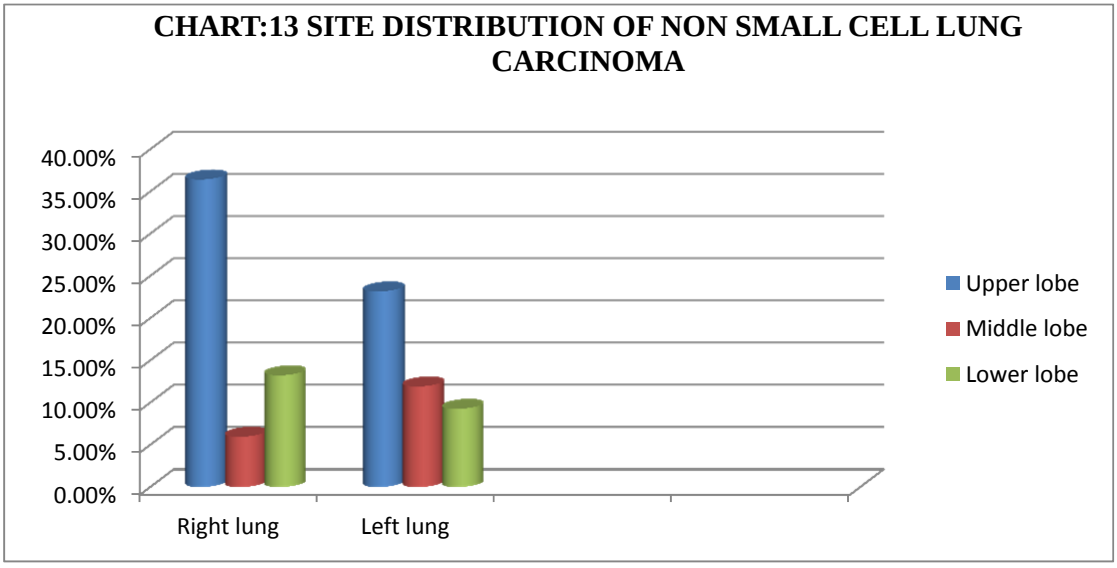


Regarding the site of prevalence of tumours out of 151 cases 84 cases were seen in the right side of the lung (55.62%) and 67 cases in the left side of the lung (44.67%).The lobar distribution was as follows: 55 cases in the right upper lobe (36.42%), 9 cases in the right middle lobe (5.96%), 20 cases in the right lower lobe (13.24%), 35cases in the left upper lobe (23.17%), 14 cases in the lingual (9.27%) and 18 cases in the left lower lobe (11.27%).

Among the 151 cases of non small cell lung carcinomas, most common location of tumour is in right upper lobe (36.42%) followed by left upper lobe (23.17%). Least common site of location is in right middle lobe(5.96%). In this study right lung was more commonly affected than left lung . TABLE:13, CHART:13

TABLE:13 SITE DISTRIBUTION OF NON SMALL CELL LUNG CARCINOMA

	Upper lobe	Middle lobe	Lower lobe	Total
Rt lung	55(36.42%)	9(5.96%)	20(13.24%)	84(55.62%)
Lt lung	35(23.17%)	18(11.92%)	14(9.27%)	67(44.37%)

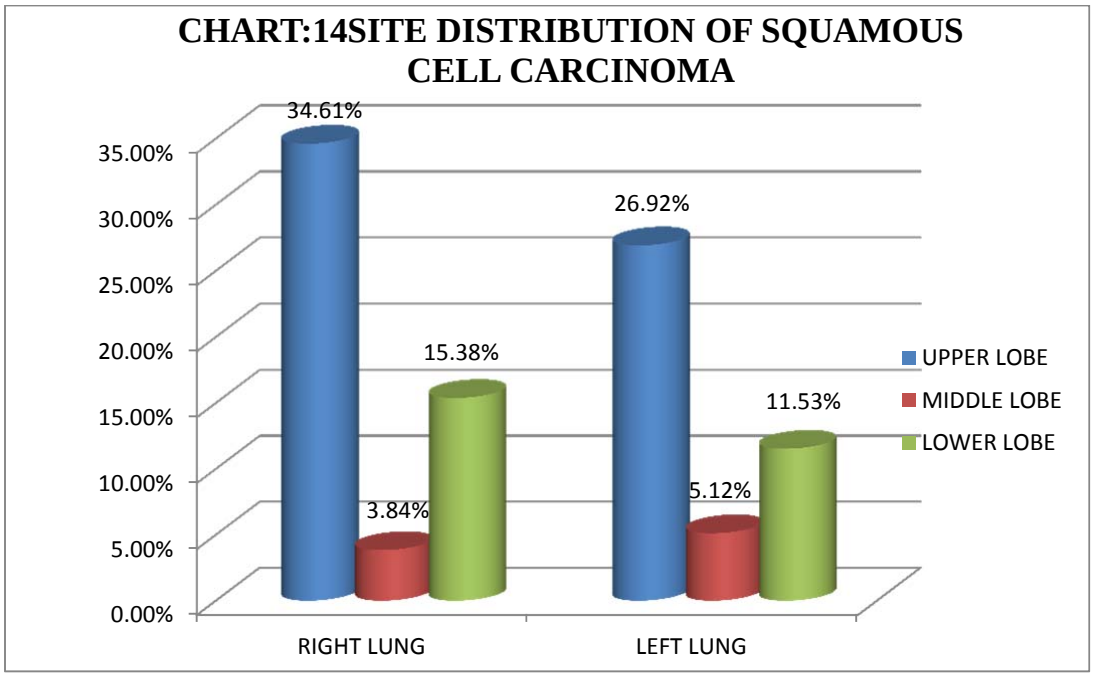


Among the 78 patients with squamous cell carcinoma , 27 cases (34.61%) were seen in right upper lobe, left upper lobe constitutes 21

(26.92%). Majority of squamous cell carcinoma were seen in right lung constituting 55.12%. Table:14 & CHART:14

TABLE:14 SITE DISTRIBUTION OF SQUAMOUS CELL CARCINOMA:

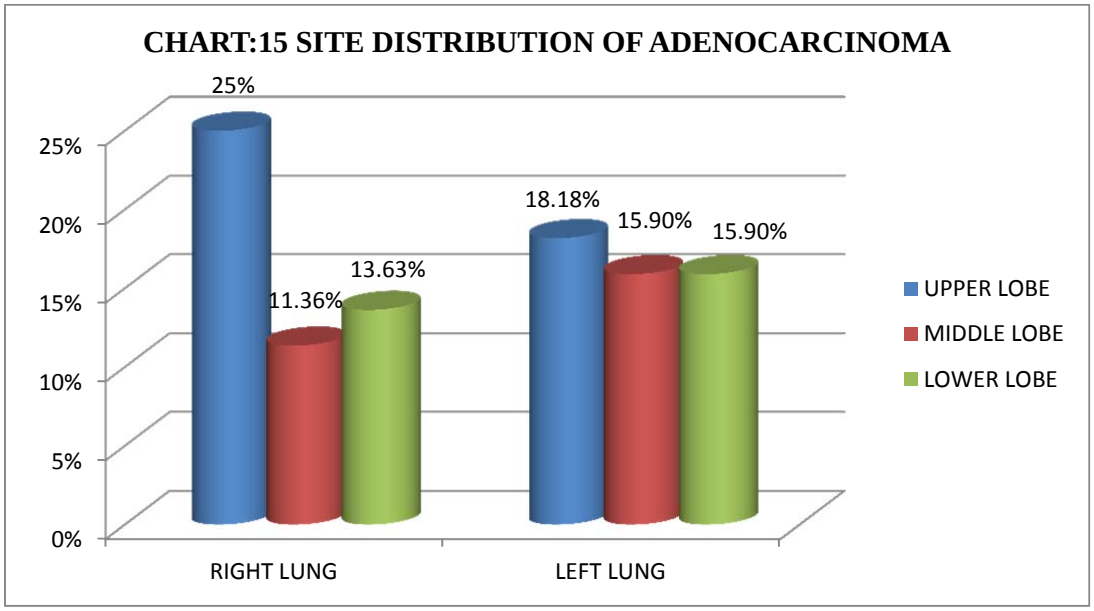
	Upper lobe	Middle lobe	Lower lobe	Total
Right lung	27(34.61%)	4(3.84%)	12(15.38%)	43(55.12%)
Left lung	21(26.92%)	5(5.12%)	9(11.53%)	35(44.87%)



Out of 44 cases of adenocarcinoma ,11 cases were seen in the right upper lobe of the lung with the percentage of 25%,followed by left upper lobe which constitutes 8 cases(18.18%).TABLE:15&CHART:15

TABLE:15 SITE DISTRIBUTION OF ADENOCARCINOMA

	Upper lobe	Middle lobe	Lower lobe
Right lung	11(25%)	5(11.36%)	6(13.63%)
Left lung	8(18.18%)	7(15.90%)	7(15.90%)

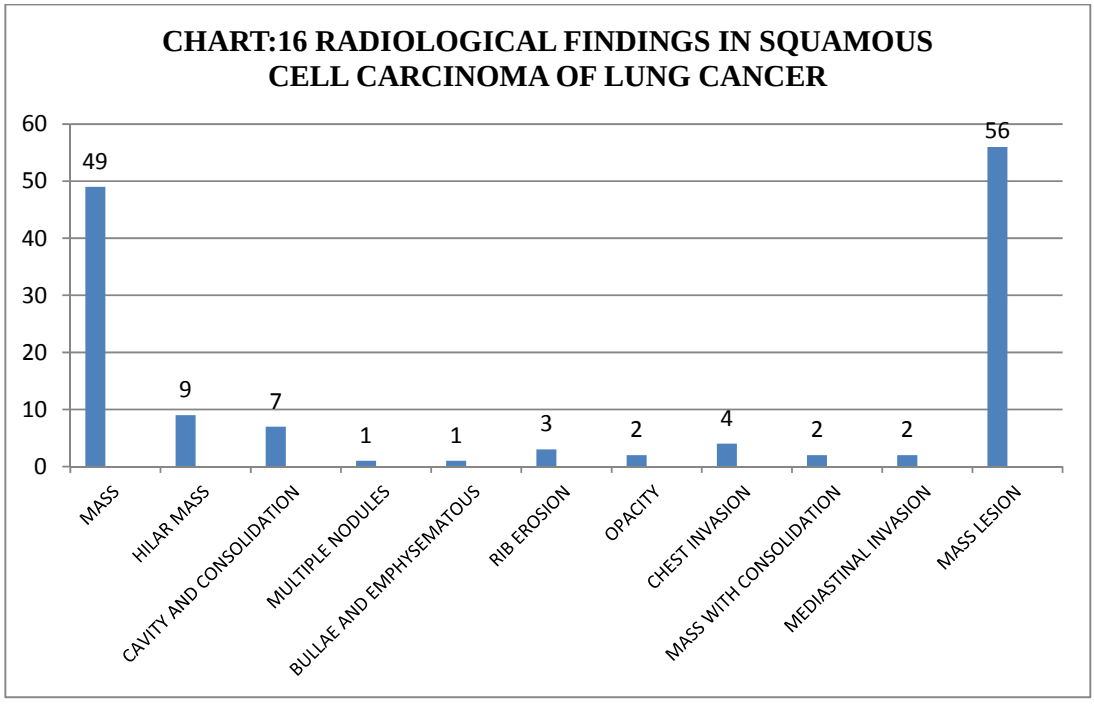


With available radiological features in this study , nodular and mass lesions are proved to be malignant . Some diagnosed as non-neoplastic lesion radiologically such as cavitatory lesion, consolidation changes, emphysematous changes etc also turned out to be malignant pathologically.

Most common radiographic finding of squamous cell carcinomas was found to be mass lesion and seen commonly as hilar mass. Other less common findings are consolidation, bullae, emphysematous lesion and cavity formation. TABLE:16 ,CHART:16

TABLE:16 VARIOUS RADIOLOGICAL FINDINGS IN SQUAMOUS CELL CARCINOMA OF LUNG CANCER

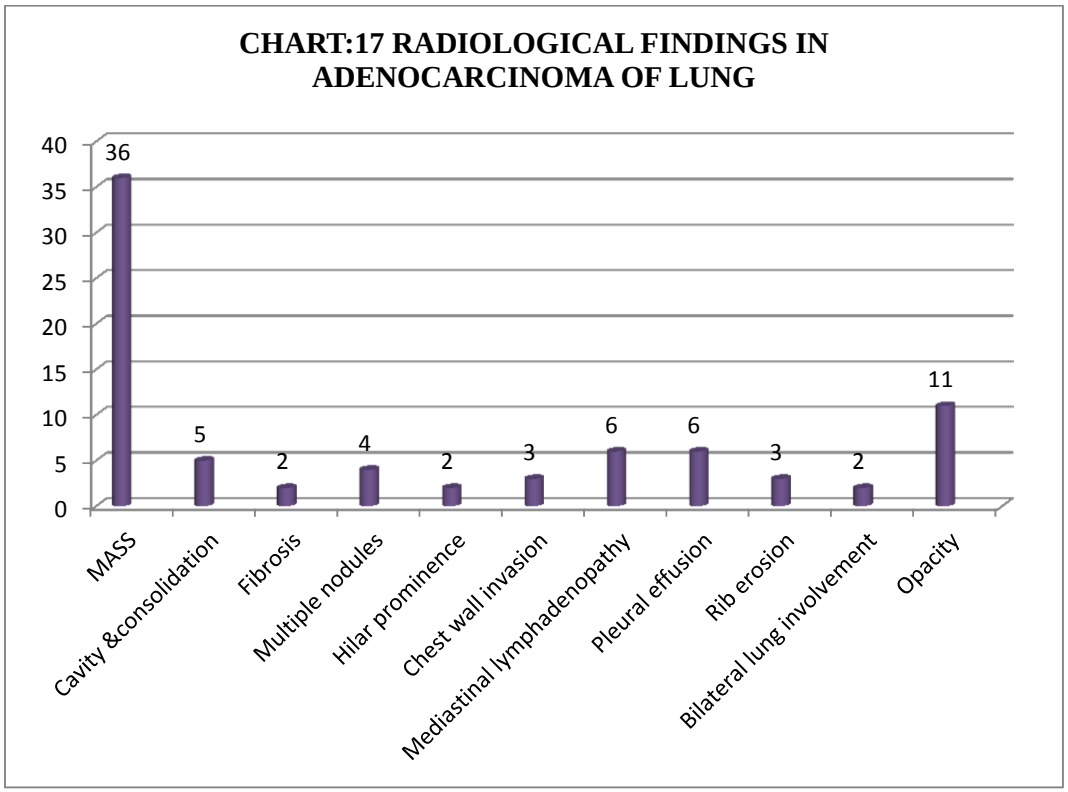
RADIOLOGICAL FINDINGS	NUMBER OF CASES
Mass	49(55.05%)
Hilar mass	9(10.11%)
Cavity and consolidation	7(7.86%)
Multiple nodules	1(1.12%)
Bullae and emphysematous changes	1(1.12%)
Rib erosion	3(3.37%)
Opacity	2(2.24%)
Chest wall invasion	4(4.49%)
Mass with consolidation	2(2.24%)
Mediastinal invasion	2(2.24%)



Most common radiological finding of adenocarcinomas was found to be mass lesion and opacity. Other less common findings are consolidation, hilar prominance and pleural effusion. TABLE:17, CHART:17

TABLE:17 VARIOUS RADIOLOGICAL FINDINGS IN ADENOCARCINOMA OF LUNG

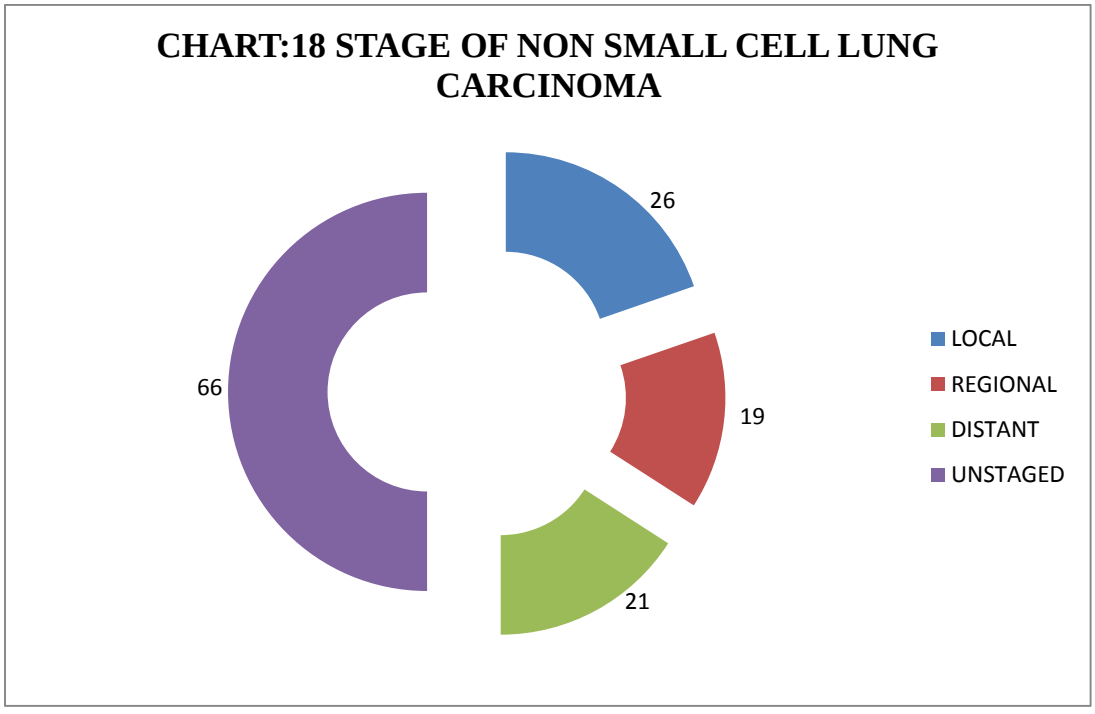
RADIOLOGICAL FEATURES	NUMBER OF CASES
Mass	36(52.17%)
Cavity &consolidation	5(7.24%)
Fibrosis	2(2.89%)
Multiple nodules	4(5.79%)
Hilar prominence	2(2.89%)
Chest wall invasion	3(4.34%)
Mediastinal lymphadenopathy	6(8.69%)
Pleural effusion	6(8.69%)
Rib erosion	3(4.34%)
Bilateral lung involvement	2(2.89%)
Opacity	11(15.94%)



With available radiological findings, local metastasis such as mediastinal, chest wall and rib invasion were seen in 26 cases(13.9%), regional metastasis (mediastinal node, cervical node, supraclavicular and scalene node metastasis) were seen in 9 cases (9.27%) and distant metastasis such as contralateral lung involvement, multiple nodules in brain ,liver and adrenal metastasis seen in 6 cases (9.93%) TABLE:18&CHART:18

TABLE:18 STAGE OF NON SMALL CELL LUNG CARCINOMA AT PRESENTATION

METASTASIS	NSCLC	PERCENTAGE
LOCAL	26	17.21%
REGIONAL	19	12.58%
DISTANT	21	13.90%
UNSTAGED	66	



Among the 78 cases of squamous cell carcinoma, local metastasis were seen in 11 cases (14.10 %), 9 cases (11.53%) showed regional metastasis and 8 cases (10.25%) showed distant metastasis .

Among the 44 cases of adenocarcinoma, 9 cases(20.45%) showed Local metastasis, Regional metastasis and Distant metastasis accounts for 5 (11.36%) and 10 (22.72%) cases respectively.

In 31 cases of poorly differentiated NSCLCs, the local, regional and distant metastasis each accounts for 2 cases (6.45%), 1 case (2.27%) and 3 cases (6.81%) respectively. Of these two, the metastasis is more common in adeno carcinoma.

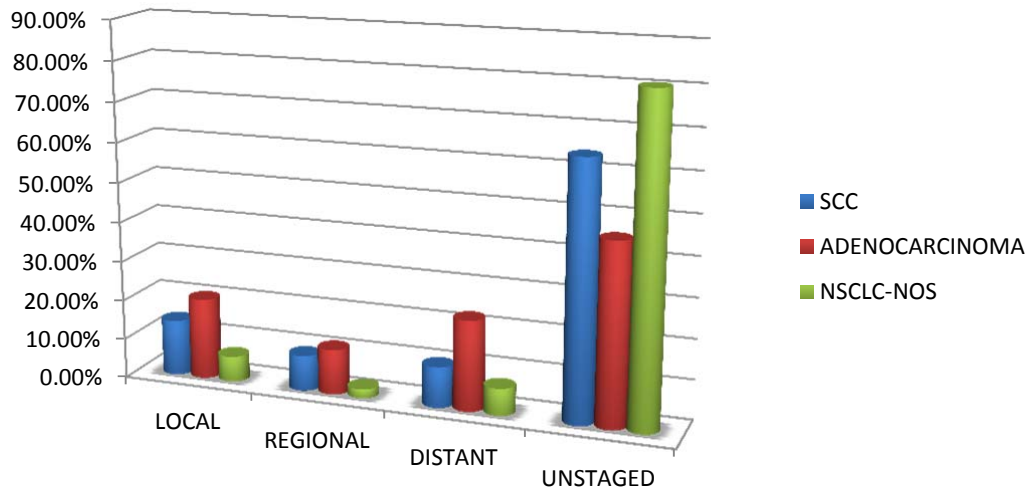
Among the 26 cases of local metastasis chest wall invasion like rib and sternum involvement were seen in 14 cases, mediastinal invasion accounts for 12 cases.

Among the 21 cases with distant metastasis, separate lesion in ipsilateral or contralateral lung were seen in 10 cases, 7 cases showed brain metastasis, 2 cases showed liver metastasis and adrenal metastasis was seen in 1 case. TABLE :19&CHART:19

TABLE : 19 STAGE OF THE TUMOUR AT PRESENTATION FOR HISTOLOGIC SUBTYPES OF NON SMALL CELL LUNG CARCINOMA

STAGE	SCC	ADENOCARCINOMA	NSCLC-NOS
LOCAL	11(14.1%)	9(20.45%)	2(6.45%)
REGIONAL	9(8.97%)	5(11.36%)	1(2.27%)
DISTANT	8(10.25%)	10(22.72%)	3(6.81%)
UNSTAGED	50(64.10%)	20(45.45%)	25(80.64%)

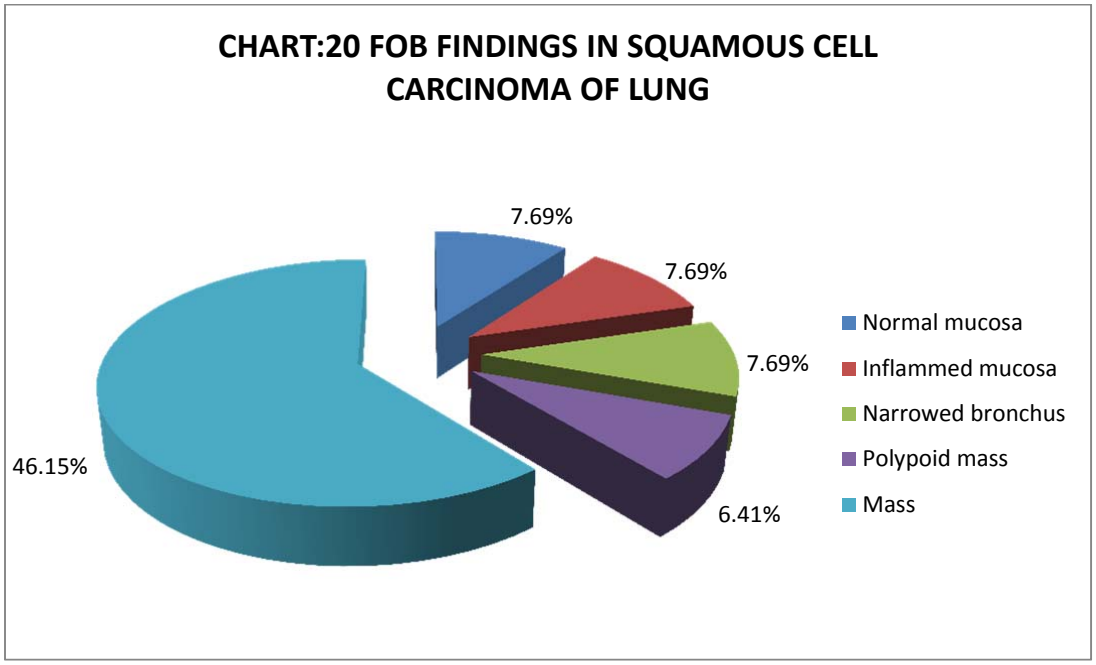
CHART:19 STAGING OF HISTOLOGICAL SUBTYPES OF NSCLC



With the available fibroptic bronchoscopic findings, both SCC and adenocarcinoma may present with or without mass and can also be seen with normal mucosa. All the polypoidal lesion identified in this study showed squamous cell carcinoma.TABLE:20&21 ,CHART:20&21

TABLE:20 FOB FINDINGS IN SCC OF LUNG

FOB Findings	Number of cases	Percentage
Normal mucosa	6	7.69%
Inflamed mucosa	6	7.69%
Narrowed bronchus	6	7.69%
Polypoid mass	5	6.41%
Mass	36	46.15%
Total	59	

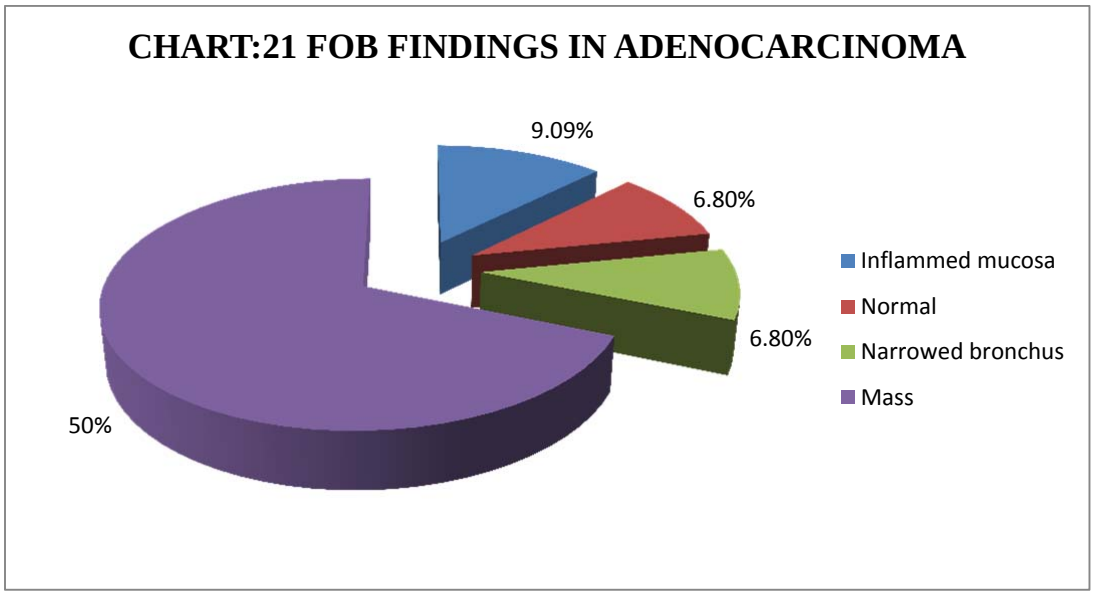


With the available FOB findings, most of the squamous cell carcinoma and adenocarcinomas are presented with endo bronchial growth.

TABLE:21 FOB FINDINGS IN ADENOCARCINOMA

FOB FINDINGS	NUMBER OF CASES	PERCENTAGE
Inflamed mucosa	4	9.09%
Normal	3	6.8%
Narrowed bronchus	3	6.8%
Mass	22	50%
Total	32	

CHART:21 FOB FINDINGS IN ADENOCARCINOMA

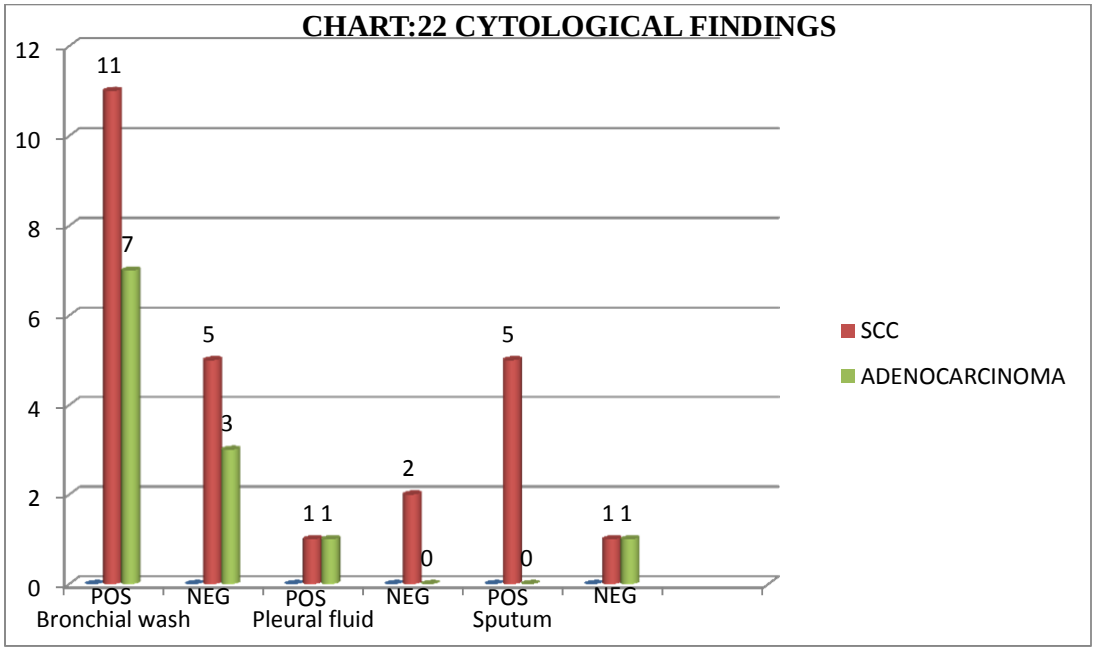


The cytological findings were available for 37 cases. Among the 37cases, 28 were bronchial wash cytology, sputum were 7 and pleural fluid were 4. Bronchial wash positive for SCC and adenocarcinoma were 11 and 7

cases respectively. Most of sputum cytology were positive for squamous cell carcinomas. TABLE :22&CHART:22

TABLE:22 CYTOLOGICAL FINDINGS

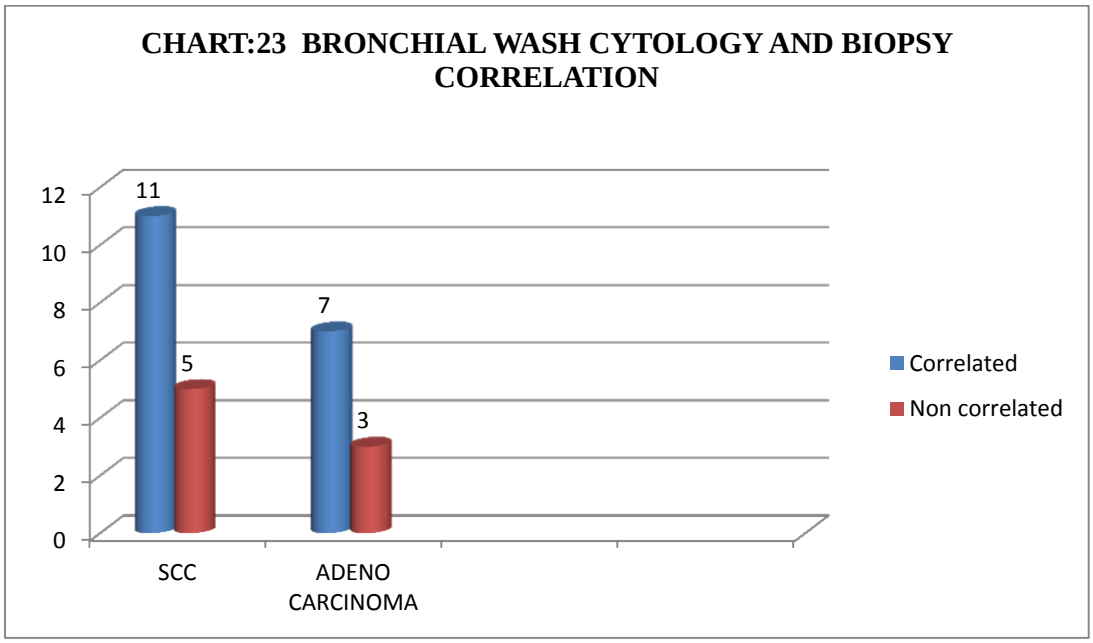
	Bronchial wash		Pleural fluid		Sputum	
	POS	**NEG**	**POS**	**NEG**	**POS**	**NEG**
SCC	11	5	1	2	5	1
ADENOCA	7	3	1	0	0	1



Among the 26 bronchial wash cytology cases,11 cases with squamous cell carcinoma and 7 cases with adenocarcinoma were correlated with biopsy with the percentage of 60.23%. TABLE:23 &CHART:23

TABLE:23 BRONCHIAL WASH CYTOLOGY AND BIOPSY CORRELATION

	Correlated	Non correlated
SCC	11	5
ADENO CA	7	3
TOTAL	18(69.23%)	8(30.76%)



FNAC from Supraclavicular lymphnode were positve in 4 squamous cell carcinoma cases and in 1 case of adenocarcinoma.

Subtyping of NSCLC-NOS was done based on the algorithm given by IASLC/ATS/ERS International multidisciplinary team such as the tumours which are positive for TTF-1 and or mucin positive as NSCLC favouring adenocarcinoma and those tumours which are positive for p40 are termed as

NSCLC favouring squamous cell carcinoma. if both TTF-1/ mucin stains and p40 are positive then the possibility of adenosquamous carcinoma should be considered. But if both TTF-1/mucin and p40 are negative and fails to show any squamous or glandular morphology, the diagnosis still remains as NSCLC-NOS. TABLE: 24

TABLE:24-SUBTYPING OF NSCLC BASED ON MUCIN STAIN AND IHC MARKERS

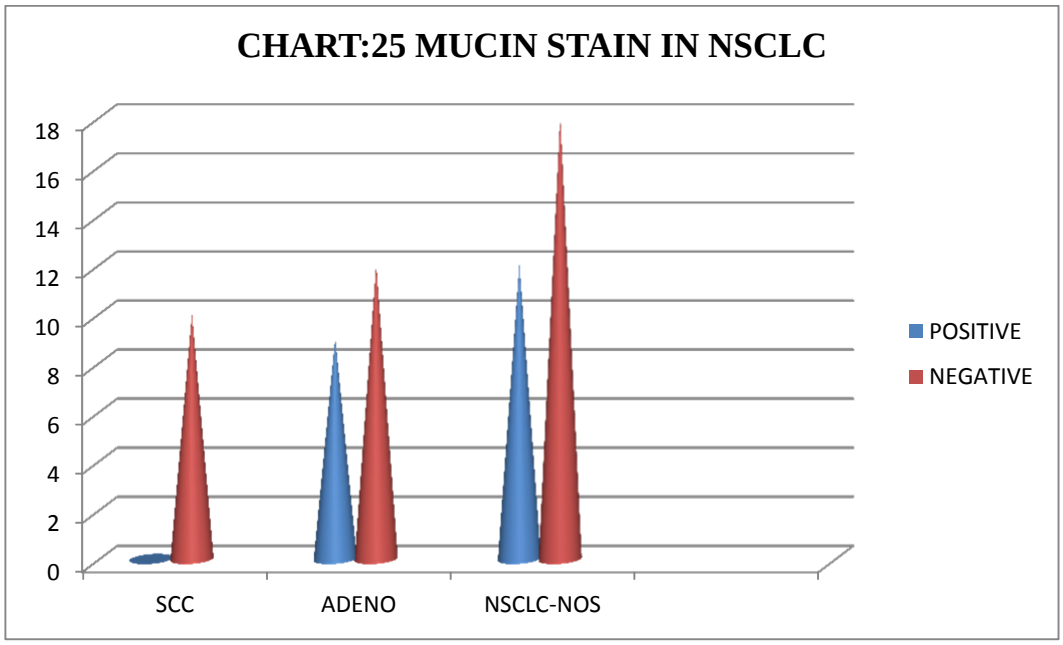
Markers	ADC	SCC	ADENOSQ	NSCLC-NOS
AB/PAS	+	-	+/-	-
TTF-1	+	-	+	-
P40	-	+	+	-

For mucin stains, in group1, 7 out of 10 adenocarcinomas were positive and in group 2, 12 out of 30 were positive. Mucin stains was negative in 3 cases of proven adenocarcinoma from group 1.TABLE:25 & CHART: 25

TABLE:25 MUCIN STAIN IN NSCLC:

	SCC	ADENO	NSCLC-NOS
POSITIVE	0	7	12
NEGATIVE	10	3	18
TOTAL	10	10	30

p value - 0.005 chi square test - 10.5

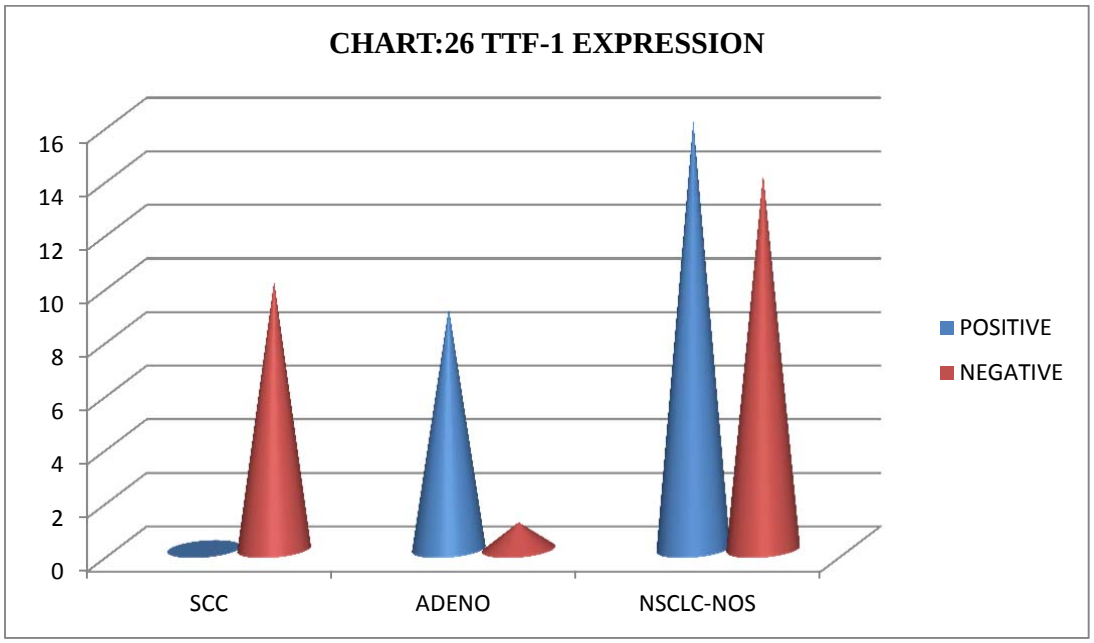


Regarding TTF-1, from group 1, 9 out of 10 cases of adenocarcinoma were positive and 16 out of 30 from group 2 were positive. TTF-1 was negative in all the poorly differentiated SCCs. TABLE:26 & CHART 26.

TABLE:26 TTF-1 EXPRESSION

	SCC	ADENO	NSCLC-NOS
POSTIVE	0	9	16
NEGATIVE	10	1	14
TOTAL	10	10	30

p value - 0.000 chi squre test - 16.5



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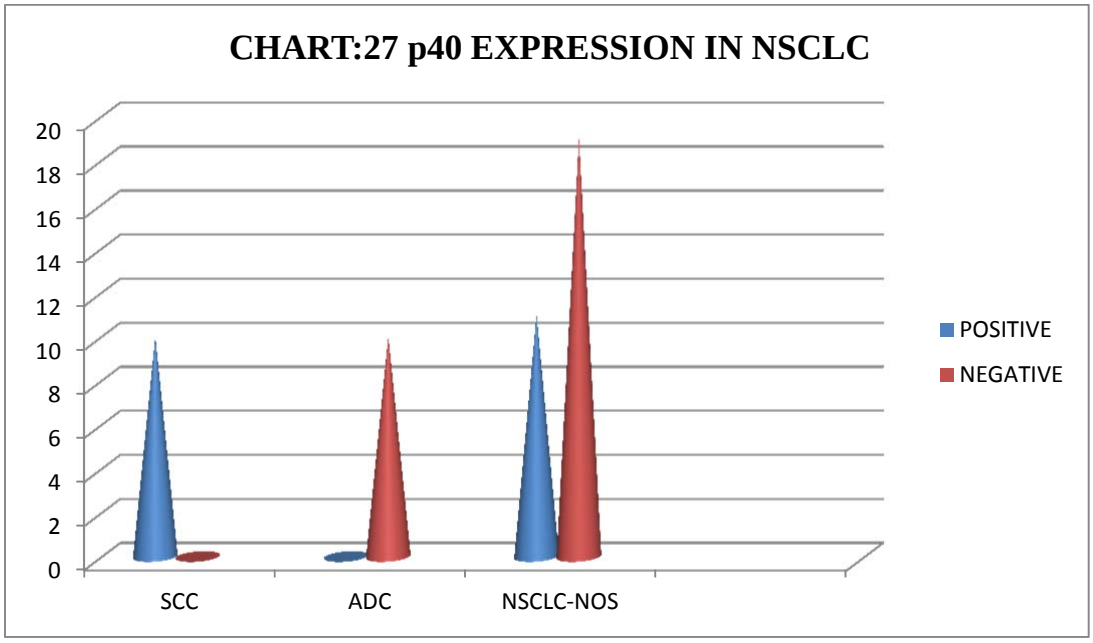
Regarding p40, in group 1, all 10 cases of squamous cell carcinoma were positive and in group 2 ,11 out of 30 cases were positive. TABLE:27 & CHART:27

TABLE:27 p40 EXPRESSION IN NSCLCs

	SCC	ADC	NSCLC-NOS
POSITIVE	10	0	11
NEGATIVE	0	10	19
TOTAL	10	10	30

p value - 0.000 chi square - 21.4



TTF-1 was expressed in 50% of the adenocarcinoma cases and p40 was positive in 42% of squamous cell carcinoma. Both markers were positive in 1 case and it was considered adenosquamous carcinoma. 6% of the tumours were negative for both markers and were considered as NSCLC-NOS. TABLE:28

TABLE:28 SUBTYPING OF NSCLCs BASED ON IHC MARKERS

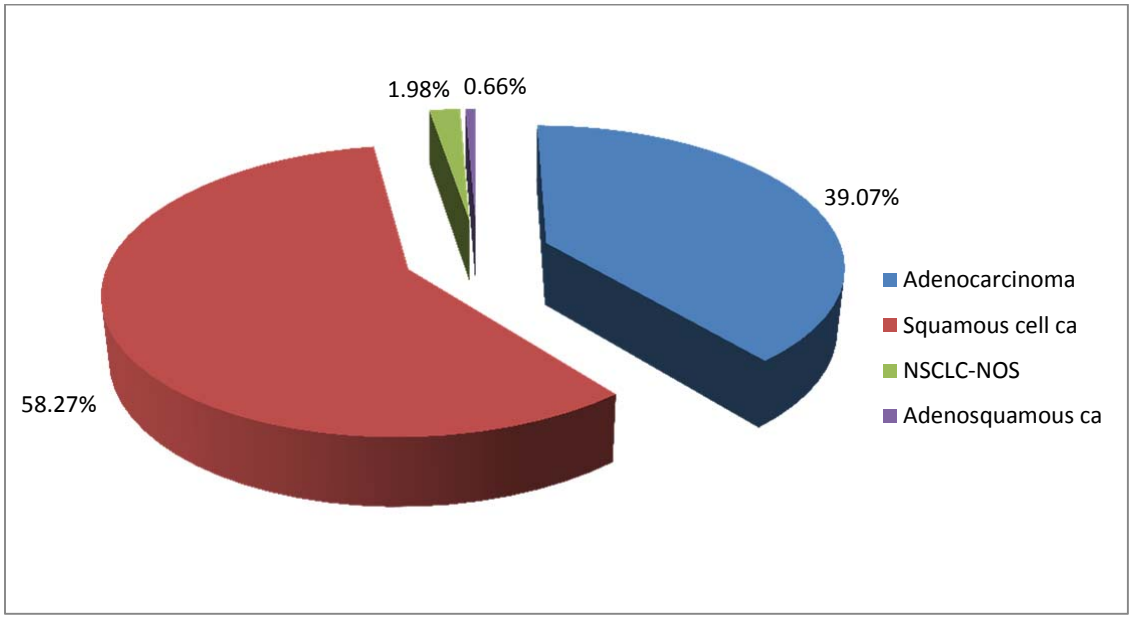
TTF-1	p40	Subtypes	Percentage	Number of cases(50)
+	-	ADC	50%	25
-	+	SCC	42%	21
+	+	ADENOSQ	2%	1
-	-	NSCLC-NOS	6%	3

According to IASLC/ATS/ERS, squamous cell carcinoma were the commonest subtype accounting for 58.27% of cases. According to WHO classification, NSCLC-NOS accounts for 19.86%. But in this study with the use of Mucin stain, TTF-1 and p40 markers, its percentage minimised into 3% because of subcategorization. **TTF-1 and mucin stain positive** cases were subtyped as **favouring adenocarcinoma** and **p40 positive** cases were subtyped as **favouring squamous cell carcinoma**. 3% of cases were **negative for both TTF-1 and p40** ,so it was still subtyped as **NSCLC-NOS** category. TABLE:29 & CHART:29

TABLE:29 SUBTYPES NON SMALL CELL LUNG CARCINOMA BASED ON IASLC/ATS/ERS CLASSIFICATION:

Subtype	Number of cases	Percentage
Adenocarcinoma	59	39.07%/
Squamous cell ca	88	58.27%
NSCLC-NOS	3	1.98%
Adenosquamous ca	1	0.66%
Total	151	

CHART:29 SUBTYPES NON SMALL CELL LUNG CARCINOMA ACCORDING TO IASLC/ATS/ERS CLASSIFICATION



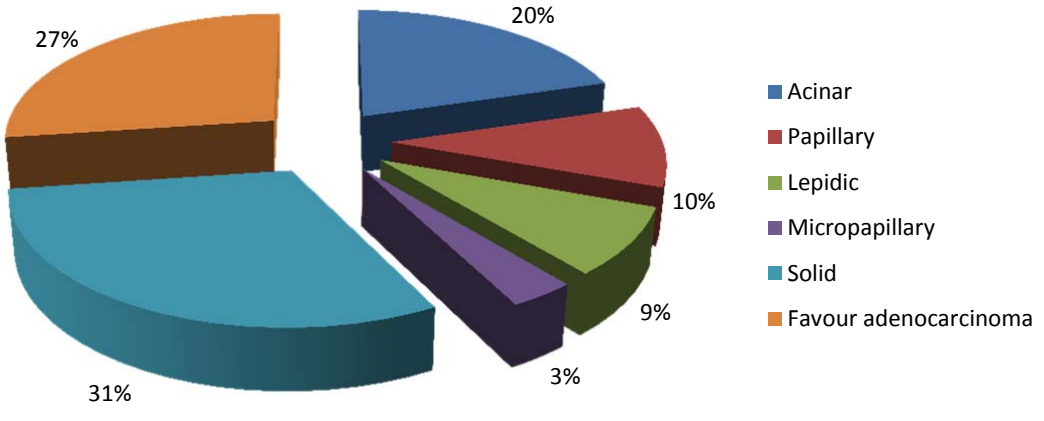
According to the classification by IASLC/ATS/ERS, most predominant type of adenocarcinoma was solid subtype which accounts for 18 cases and next common subtype was acinar which constitutes 12 cases. Other subtype

like lepidic predominant type of adenocarcinoma accounts for 5 cases, micropapillary pattern constitutes 2 cases and papillary pattern was 6 cases. TABLE:30&CHART:30

TABLE:30 ADC SUBTYPES BASED ONIASLC/ATS/ERS CLASSIFICATION

Subtypes	Number of cases	Percentage
Acinar	12	20.33%
Papillary	6	10.16%
Lepidic	5	8.4%
Micropapillary	2	3.38%
Solid	18	30.50%
Favour adenocarcinoma	16	27.11%
Total	59	

CHART: 30 ADENOCARCINOMA SUBTYPES BASED ON IASLC/ATS/ERS CLASSIFICATION



According to WHO classification,mixed subtype was constituted 62.79%.It was further subtyped according to the presence of predominant pattern into cinar (46.15%), papillary(19.23%), lepidic(3.84%), micropapillary (7.69%), solid type.(23.07%) TABLE:31

TABLE:31 SUBTYPING OF MIXED TYPE ACCORDING IASLC/ATS/ERS CLASSIFICATION

2004 WHO classification	IASLC/ATS/ERS
MIXED SUBTYPE 26(62.79%)	Acinar subtype 12(46.15%)
	Papillary subtype 5(19.23%)
	Lepidic subtype1(3.84%)
	Solid subtype 6(23.07%)
	Micropapillary subtype 2(7.69%)

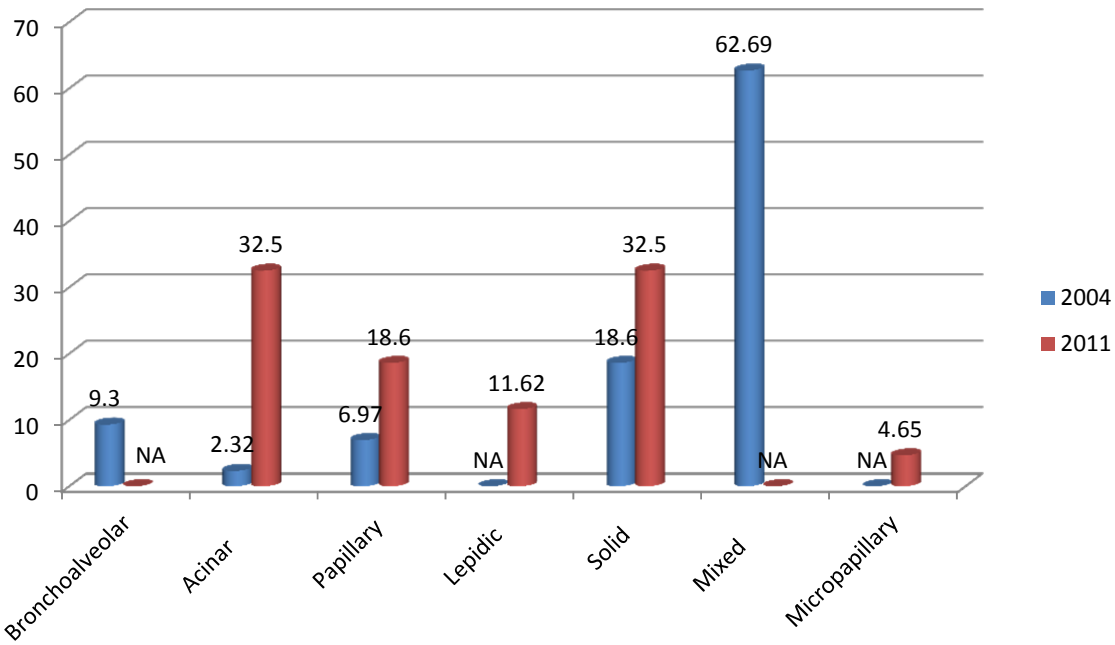
According to this study, on comparing WHO classification and IASLC/ATS/ERS 2011 classification,

In the new classification , lepidic pattern adenocarcinoma was used instead of bronchoalveolar carcinoma -non mucinous subtype (3.31%) of WHO classification and 2 cases of micropapillary carcinoma were detected which is not included in the WHO classification.TABLE:32

TABLE:32 COMPARISION OF WHO CLASSIFICATION AND IASLC/ATS/ERS CLASSIFICATION IN SUBTYPING OF ADENOCARCINOMA

2004 World health organization classification (n=43)	2011 classification by Travis et al (n=43)
Bronchoalveolar - 3(9.30%) Non mucinous	Not applicable Lepidic predominant adenoca-3(9.30%)
Bronchoalveolar -Mucinous - 2(4.54%)	Mucinous adenoca-Lepidic -1(2.32%) Acinar -1(2.32%)
Acinar -1(2.32%)	Acinar 13(32.5%)
Papillary - 3(6.97%)	Papillary 8(18.60%)
Solid -8(18.60%)	Solid 14(32.5%)
Mixed -27(62.79%)	Not applicable
Not applicable	Micropapillary 2(4.65%)

CHART:32 COMPARISION WHO CLASSIFICATION AND IASLC/ATS/ERS CLASSIFICATION



According to IASLC classififcation, with the use of AB/PAS stain,TTF-1 and p40, 30 cases of non small cell lung carcinoma-not otherwise specified type were subtyped into favouring adenocarcinoma which constitutes 50% (n=15), favouring squamous cell carcinoma which constitutes 36.66% (n=11) of cases, possible adenosquamous carcinoma were 3.33% (n=1) and unsubtyped were 10%(n=3).TABLE:33

TABLE:33 SUBTYPING OF NSCLC-NOT OTHERWISE SPECIFIED

2004 WHO classification	IASLC/ATS/ERS
NSCLC-NOS(30 Cases)	Favouring adenocarcinoma-15(50%)
	Favouring squamous cell carcinoma-11(36.66%)
	Possible Adenosquamous -1(3.33%)
	NSCLC-NOS-3(10%)

Based on this study, on comparing WHO classification and IASLC/ATS/ERS 2011 classification,

- Squamous cell carcinoma was the commonest type identified.
- Based on WHO classification , NSCLC- NOS category constituted 19.86%. of total but according to new international multidisciplinary classification with the use of mucin stains ,TTF-1 and p40 not otherwise specified category was minimised to 1.86%. In new classification ,it was further subtyped into favoring adenocarcinoma, favoring squamous cell carcinoma ,possible adenosquamous cell carcinoma.
- Among the total 151 cases of NSCLCs ,Regarding adenocarcinoma, instead of mixed subtype (17.85%) of WHO classification the presence of predominant pattern is used in new classification as

lepidic (1.32%), acinar (7.84%), papillary (3.31%), micropapillary (1.32%), and solid (3.97%) subtypes .

- In the new classification , lepidic pattern adenocarcinoma (1.98%) was used instead of bronchoalveolar carcinoma -non mucinous subtype (1.98%) of WHO classification.

- 2 cases of micropapillary carcinoma were detected which is not included in the WHO classification.

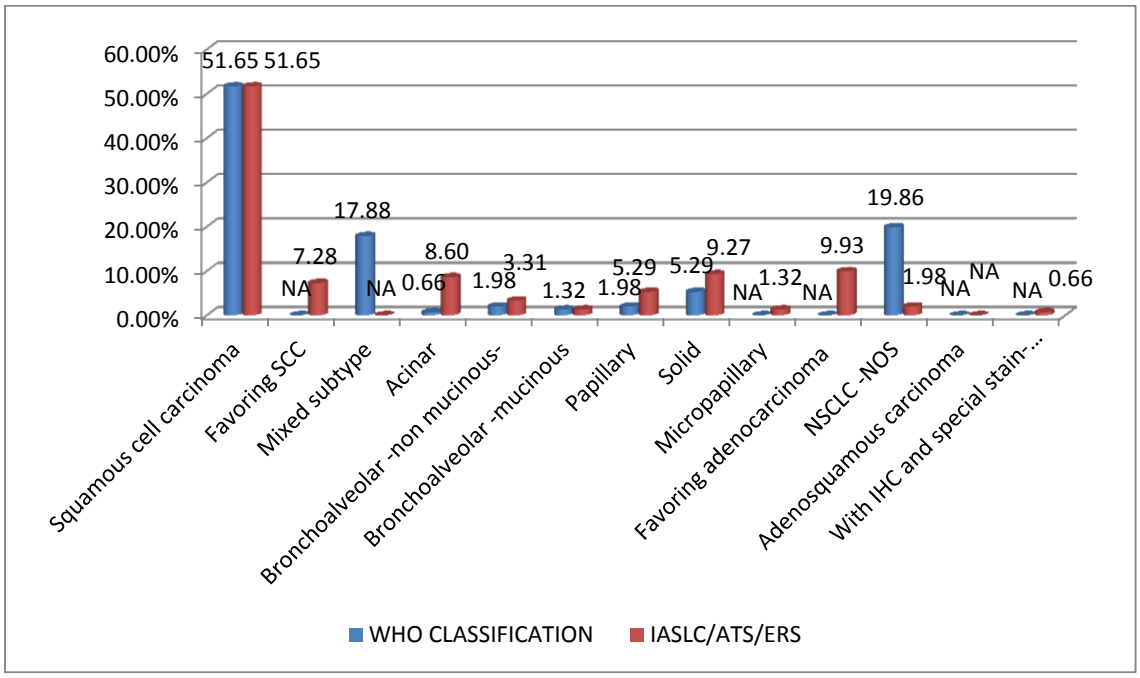
- 1 case of large cell carcinoma and sarcomatoid carcinoma each diagnosed according to WHO classification, were grouped into NSCLC -NOS in new classification. TABLE:34 &CHART :34

TABLE: 34 COMPARISION OF 2004 WHO CLASSIFICATION AND IASLC/ATS/ERS CLASSIFICATION

WHO CLASSIFICATION	IASLC/ATS/ERS
Squamous cell carcinoma -51.65%	Squamous cell carcinoma-51.65%
Not included in 2004 WHO	Favoring SCC-7.28%
Adenocarcinoma	Adenocarcinoma
Mixed subtype-17.88%	Not included
Acinar -0.66%	Acinar-8.60%
Bronchoalveolar -non mucinous-1.98%	Lepidic predominant adenocarcinoma-3.31%
Bronchoalveolar -mucinous-1.32%	Mucinous adenocarcinoma-1.32%

WHO CLASSIFICATION	IASLC/ATS/ERS
Papillary -1.98%	Papillary-5.29%
Solid -5.29%	Solid -9.27%
Not included	Micropapillary-1.32%
Not included in 2004 WHO classification	Favoring adenocarcinoma with use of IHC and special stain 9.93%
NSCLC -NOS 19.86%	NSCLC NOS-1.98%
Adenosquamous carcinoma-0	Adenosquamous carcinoma-0
Not included	With IHC and special stain-possible adenosquamous-0.66%
Large cell carcinoma-0.54%	Non small cell carcinoma -NOS - 0.54%
Sarcomatoid carcinoma-0.54%	Poorly differentiated NSCLC with spindle &/or giant cell carcinoma-0.54%

CHART:34 COMPARISION OF 2004 WHO CLASSIFICATION AND IASLC/ATS/ERS CLASSIFICATION



Colour Plates

SQUAMOUS CELL CARCINOMA

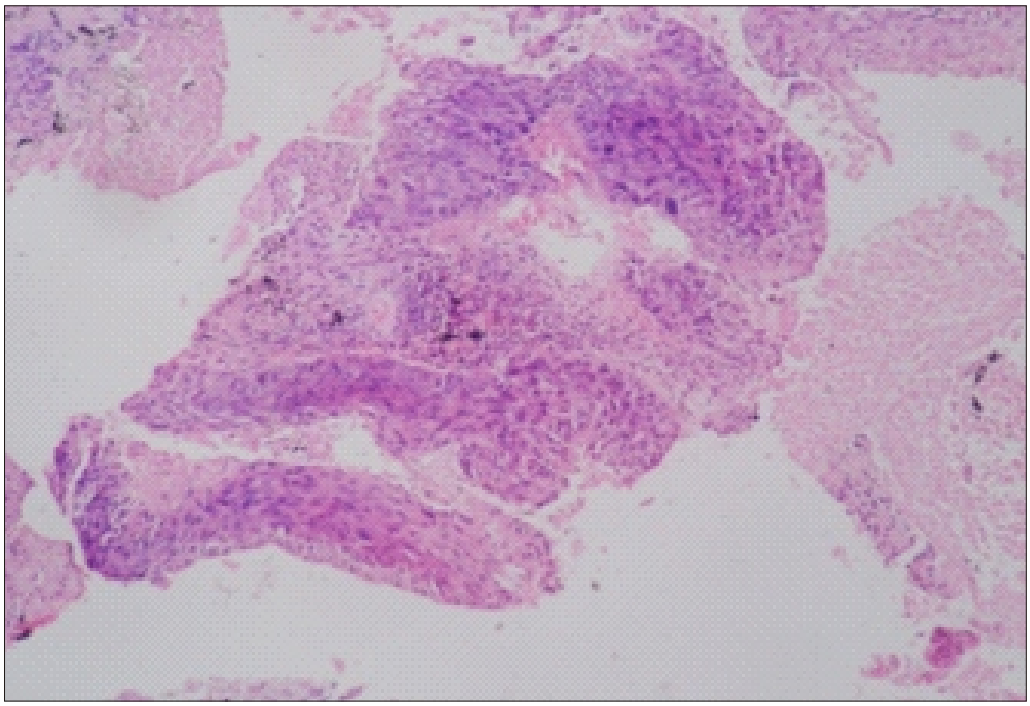


FIGURE:1 Well diffentiated squamous cell carcinoma
100X HPE NO:60/14

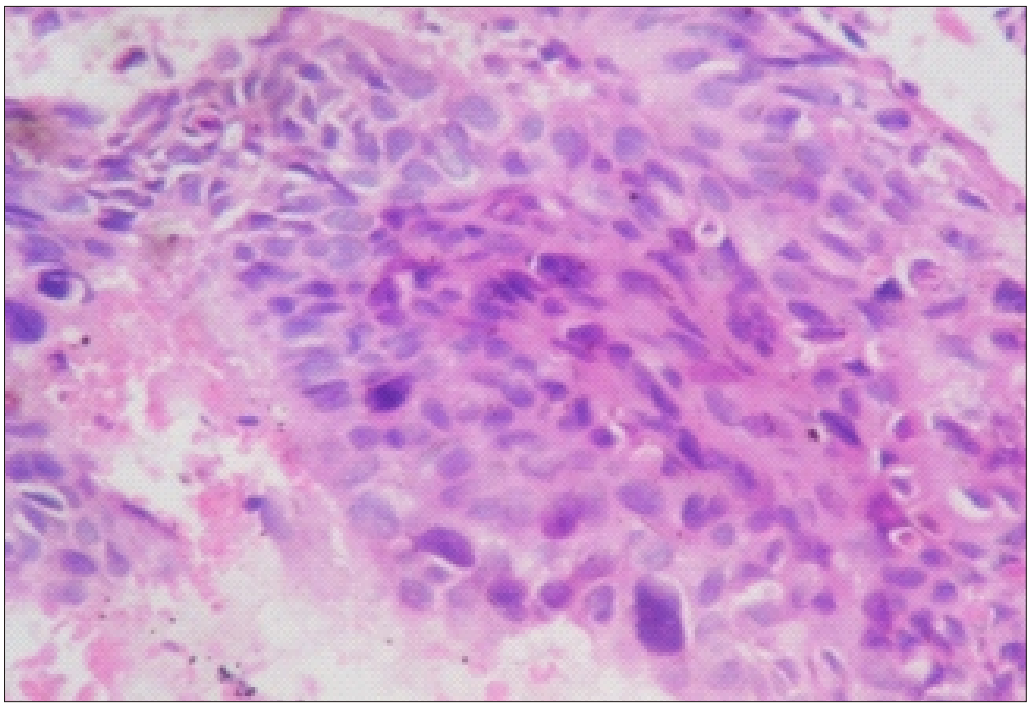


FIGURE:2 Well diffentiated squamous cell carcinoma
400X HPE NO:60/14

POORLY DIFFERENTIATED SQUAMOUS CELL CARCINOMA

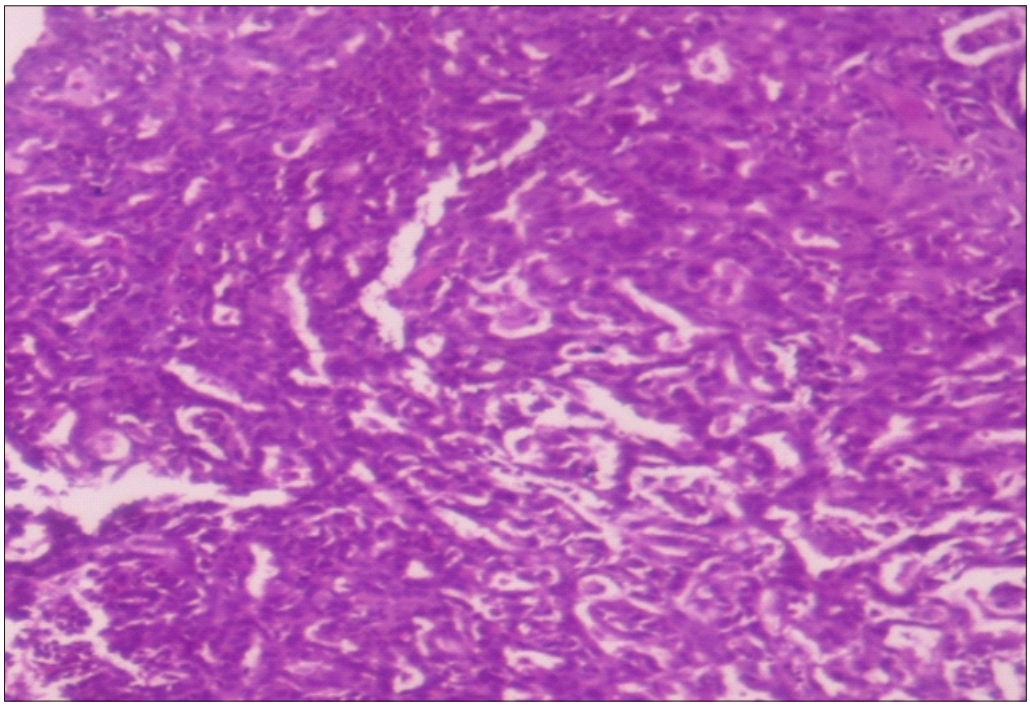


FIGURE:3 Poorly differentiated squamous cell carcinoma
100X HPE NO:1594/14

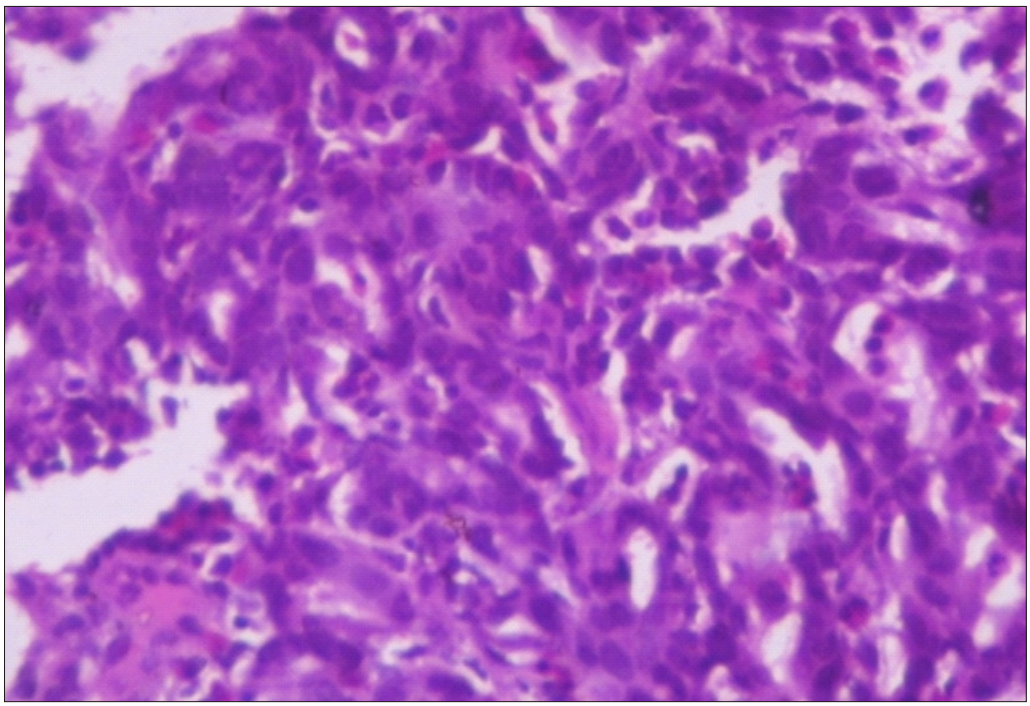


FIGURE:4 Poorly differentiated squamous cell carcinoma
400x HPE NO:1594/14

ADENOCARCINOMA WITH MIXED PATTERN

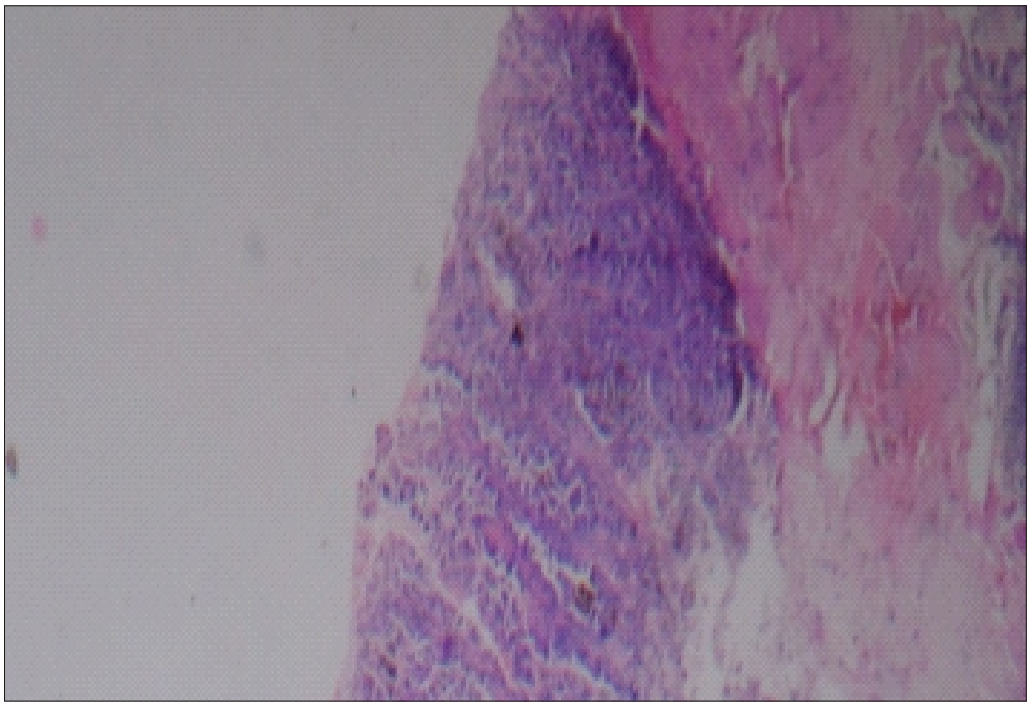


FIGURE:5 Adenocarcinoma –mixed type (Papillary and solid pattern)
100x HPE NO:2618/14

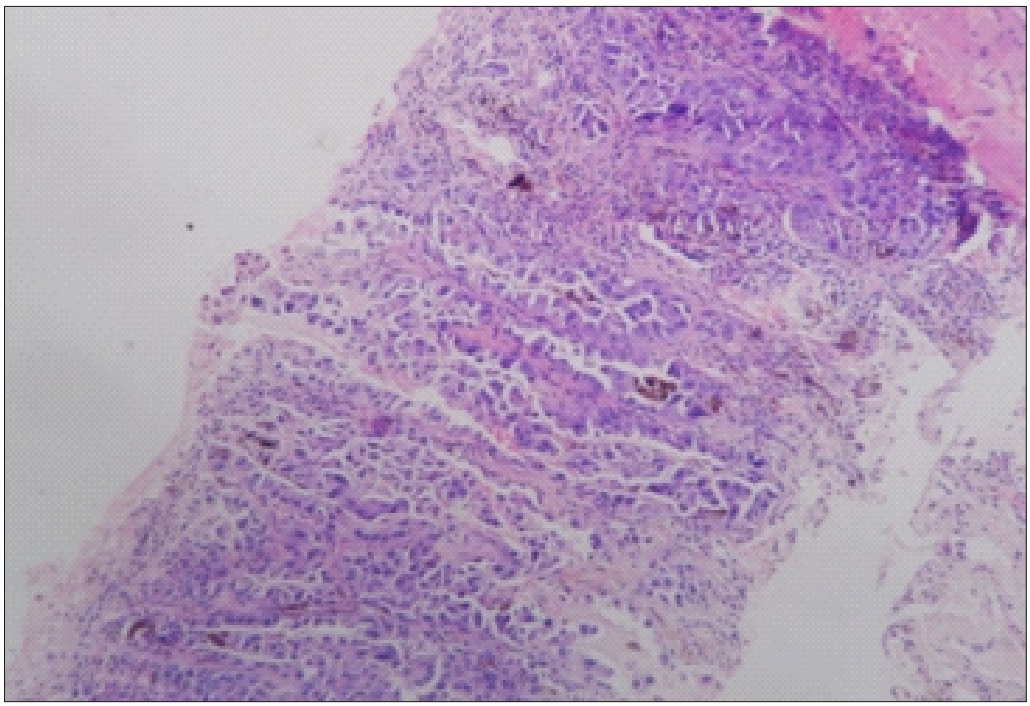


FIGURE:6 Adenocarcinoma –mixed type (Papillary and solid pattern)
400x HPE NO:2618/14

ADENOCARCINOMA –MIXED TYPE

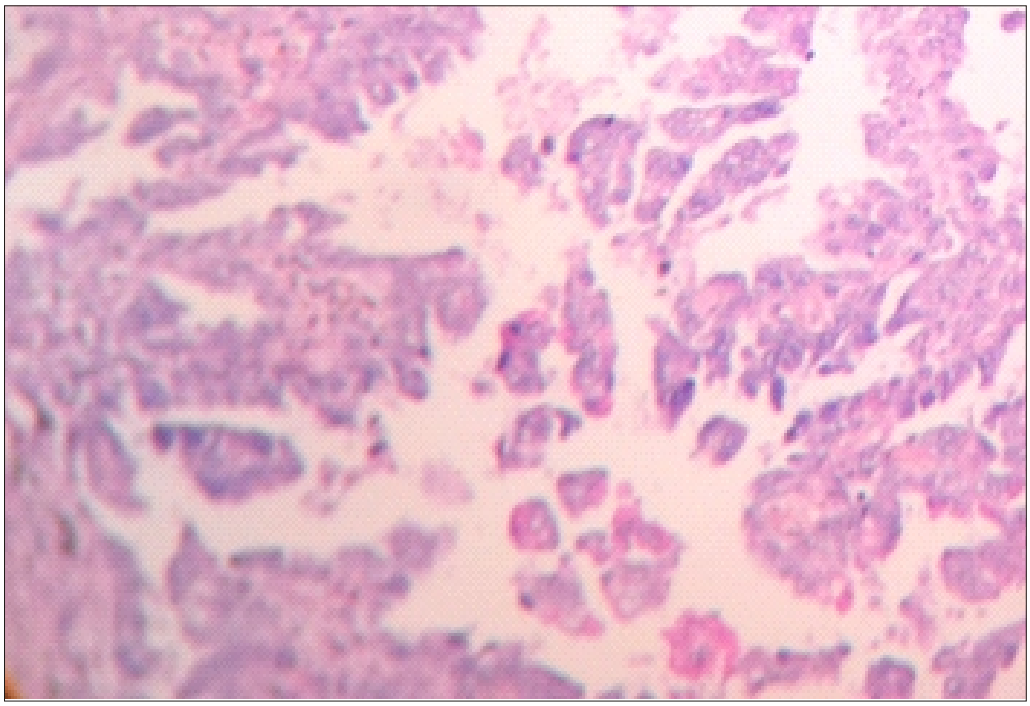


FIGURE :7 Adenocarcinoma –mixed type (papillary and micropapillary)
100x HPE NO:4308/13

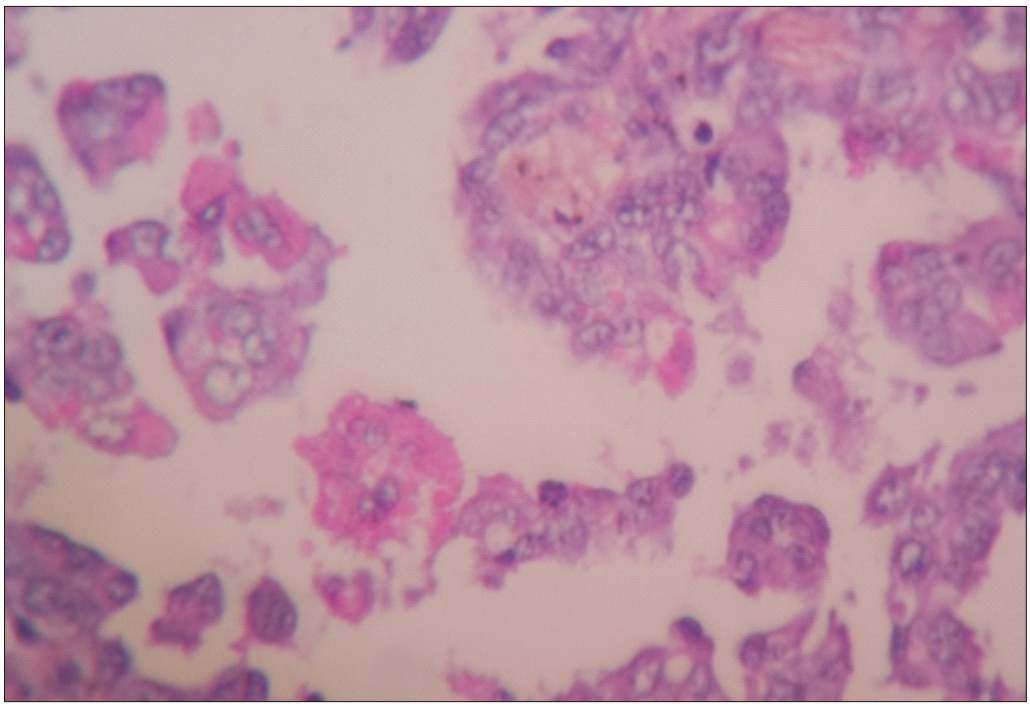


FIGURE :8 Adenocarcinoma –mixed type papillary and micropapillary)
400x HPE NO:4308/13

ADENOCARCINOMA –ACINAR PATTERN

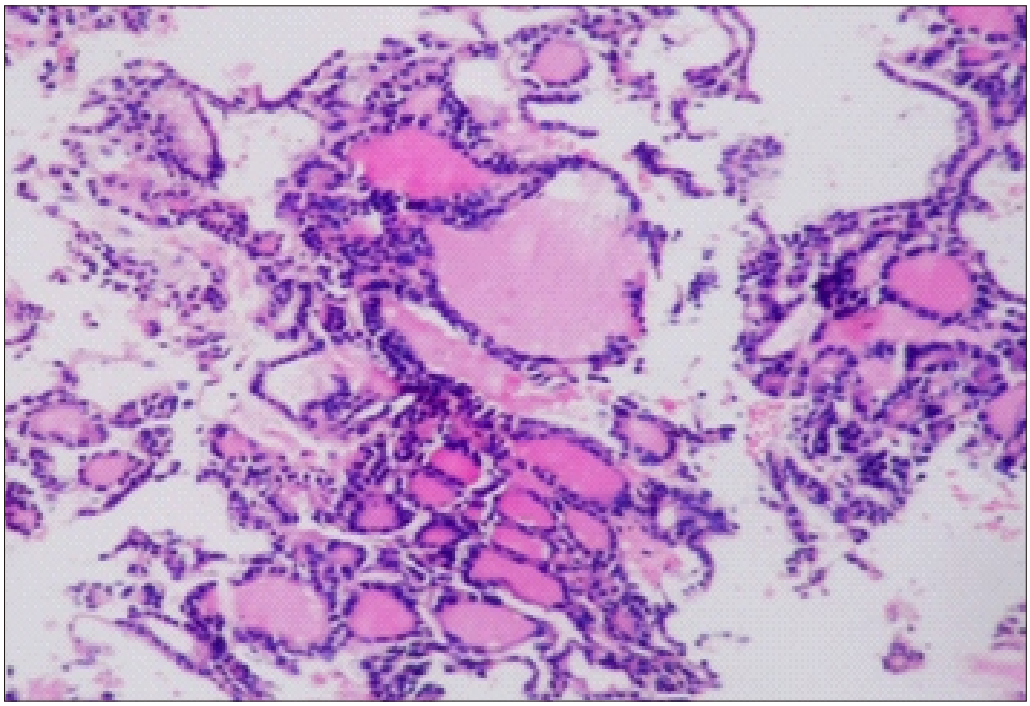


FIGURE:9 Adenocarcinoma with acinar pattern
100x HPE NO:11378/13

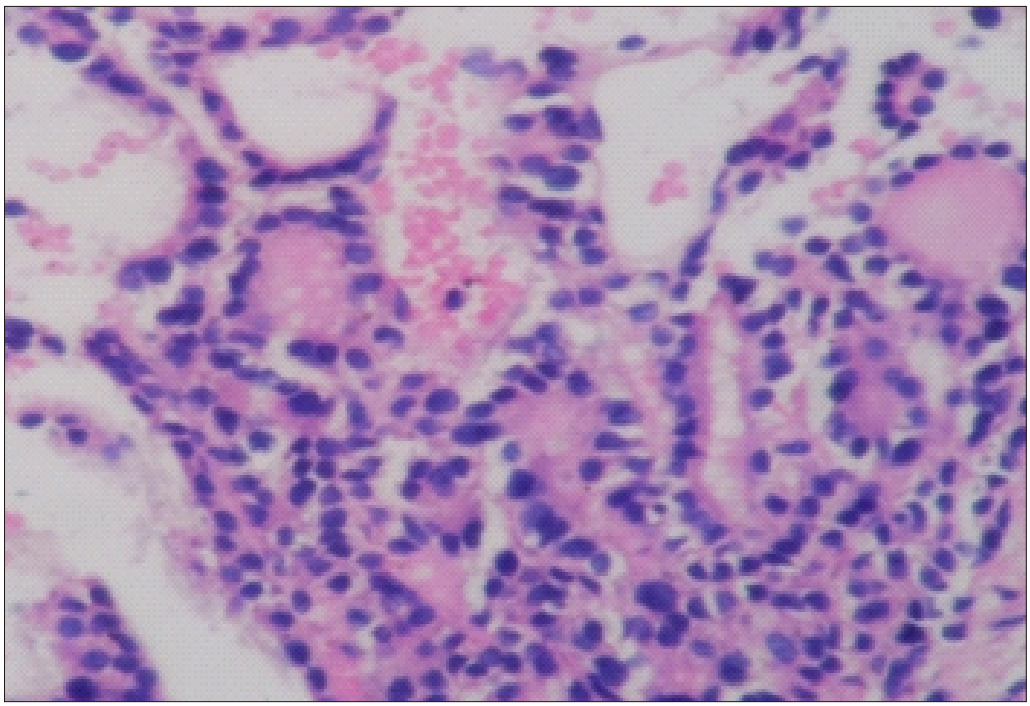


FIGURE:10 Adenocarcinoma with acinar pattern
400x HPE NO:11378/13

ADENOCARCINOMA-LEPIDIC PATTERN

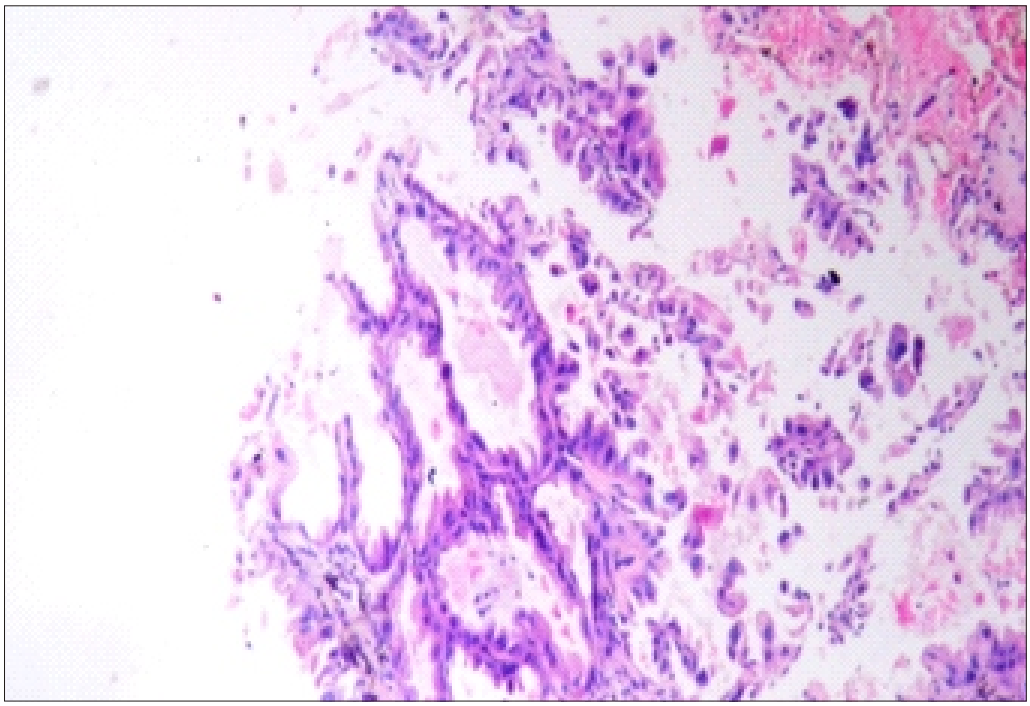


FIGURE:11 Adenocarcinoma –lepidic pattern
100X HPE NO:10058/13

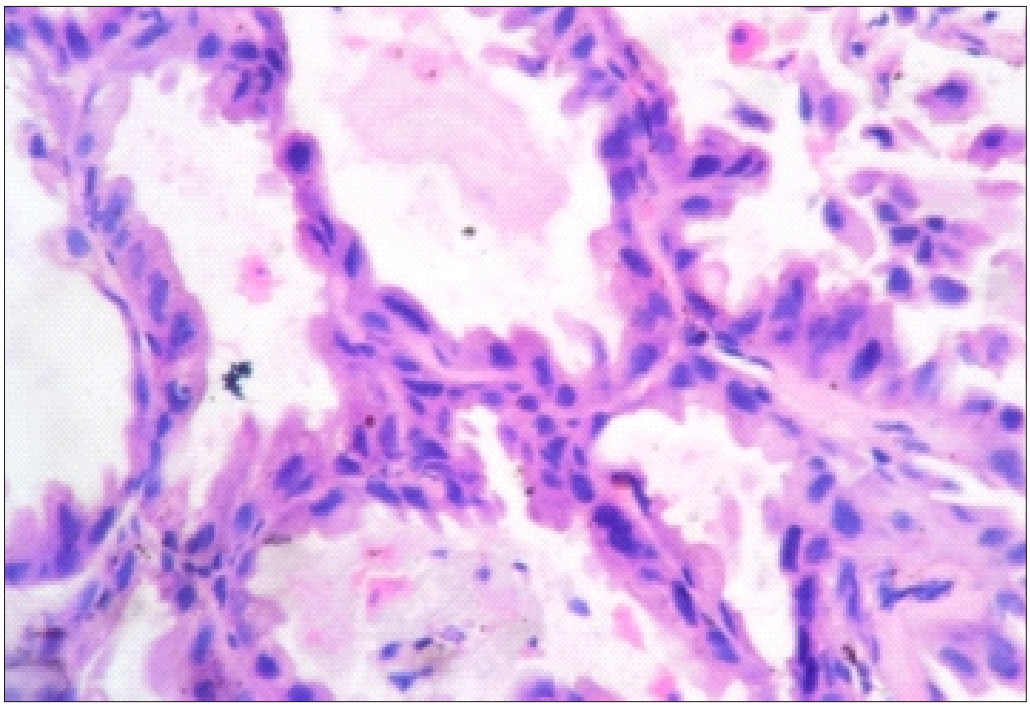


FIGURE:12 Adenocarcinoma –lepidic pattern
400X HPE NO:10058/13

ADENOCARCINOMA –PAPILLARY PATTERN

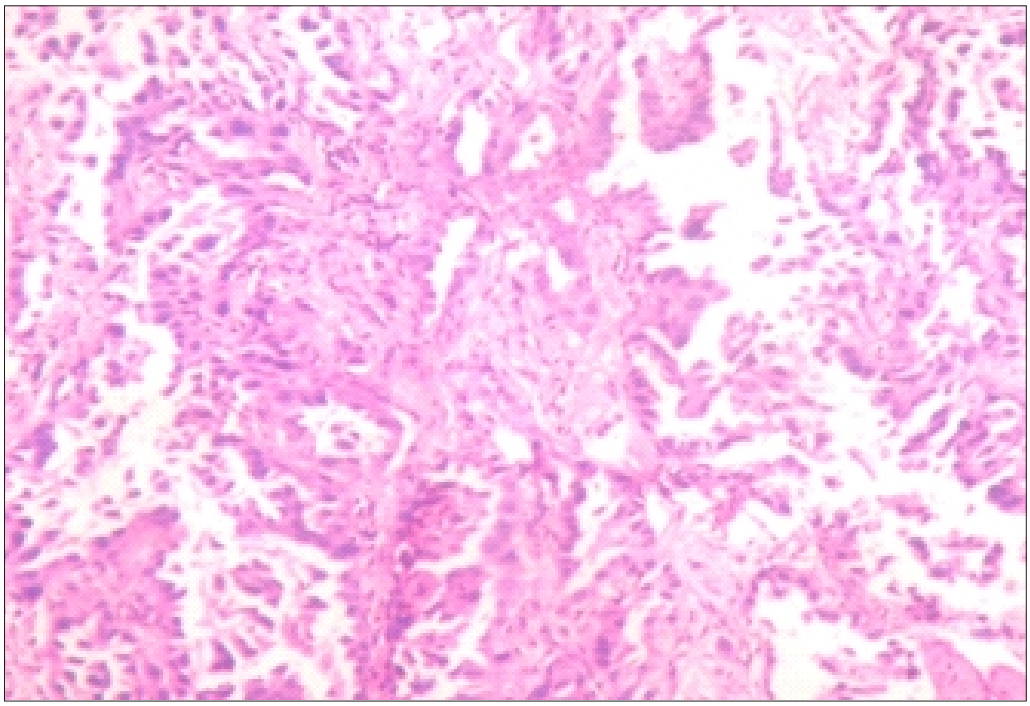


FIGURE :13 Adenocarcinoma –papillary type
100X HPE NO:6679/13

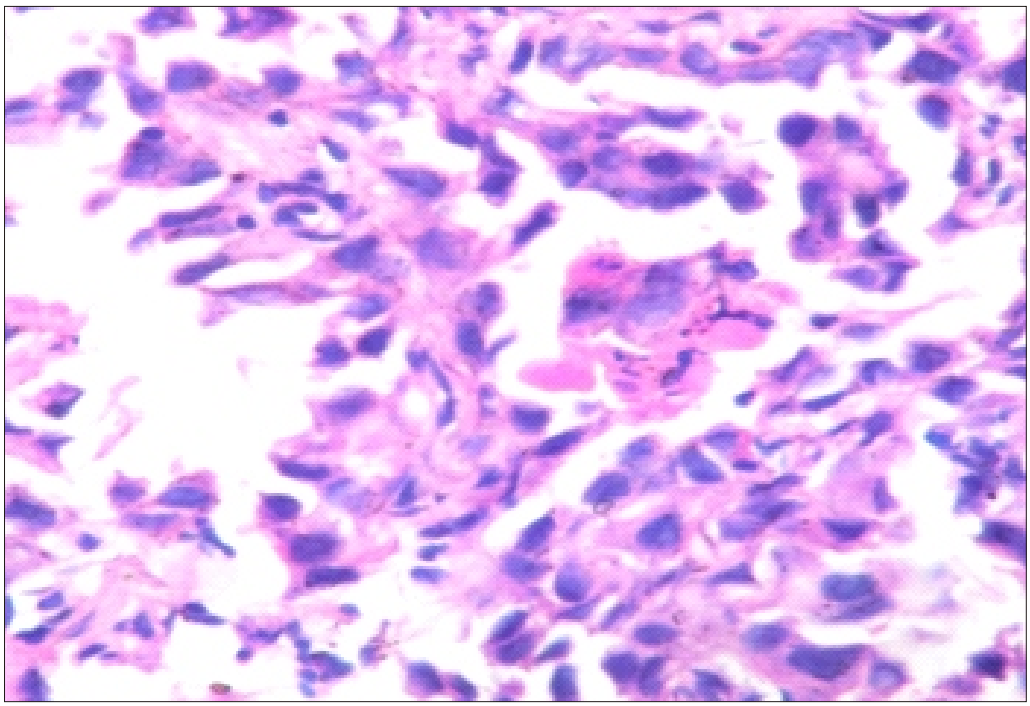


FIGURE:14 Adenocarcinoma –papillary type
400x HPE NO:6679/13

ADENOCARCINOMA –SOLID TYPE

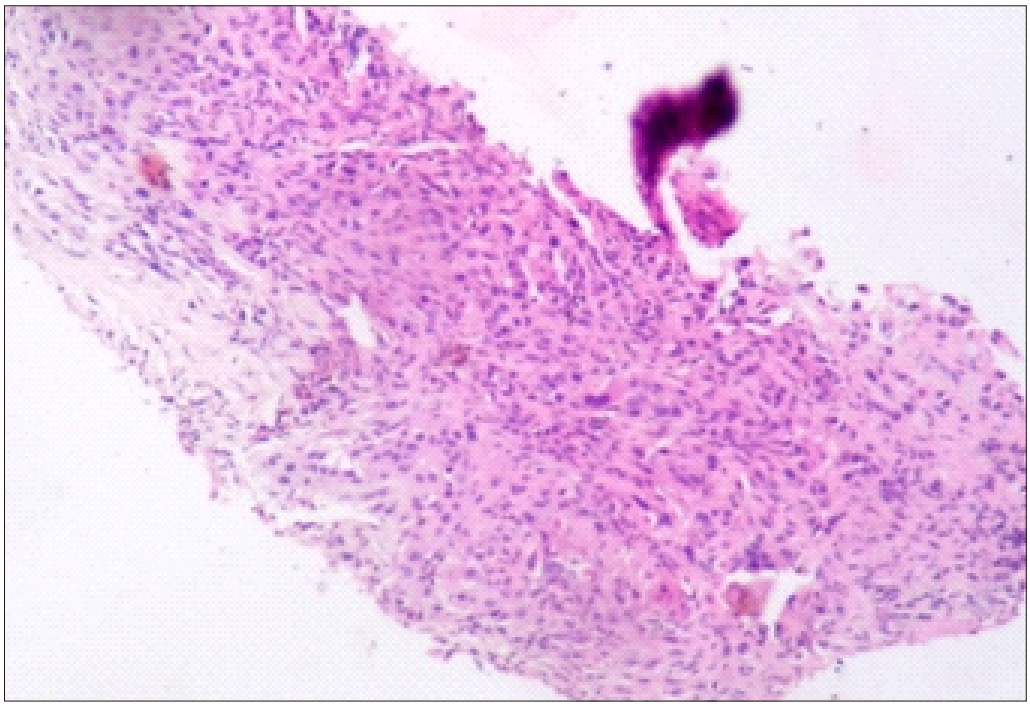


FIGURE:15 Adenocarcinoma –solid type
100X HPE NO:5527/14

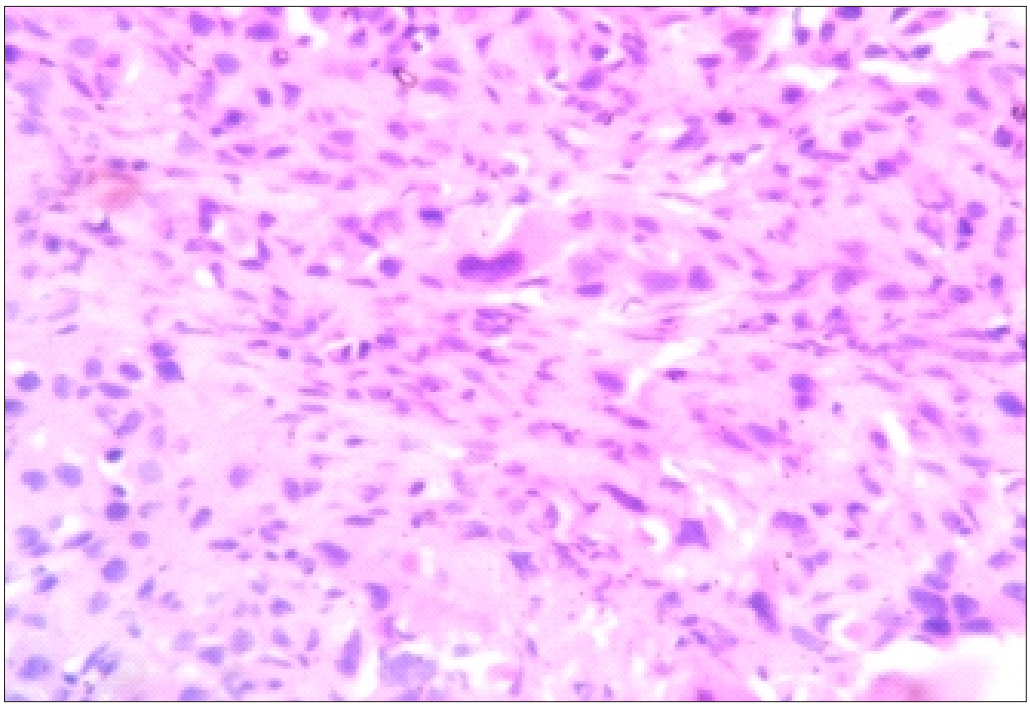


FIGURE:16 Adenocarcinoma –solid type
400X HPE NO:5527/14

ADENOCARCINOMA-MUCINOUS TYPE

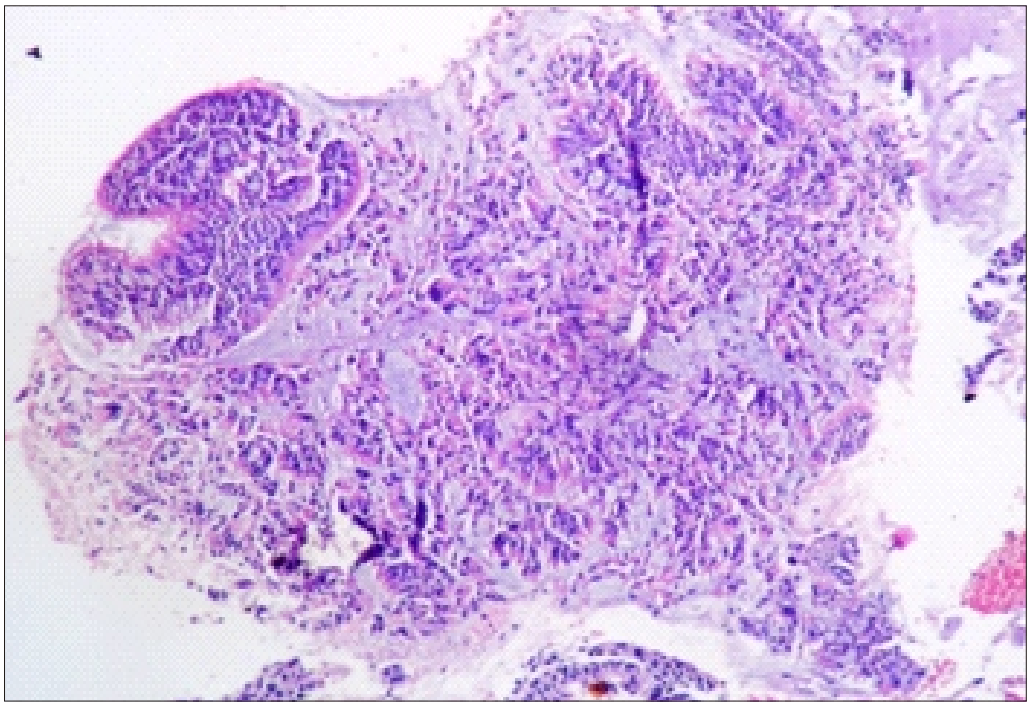


FIGURE:17 Adenocarcinoma –Mucinous type
100X HPE NO:7491/13

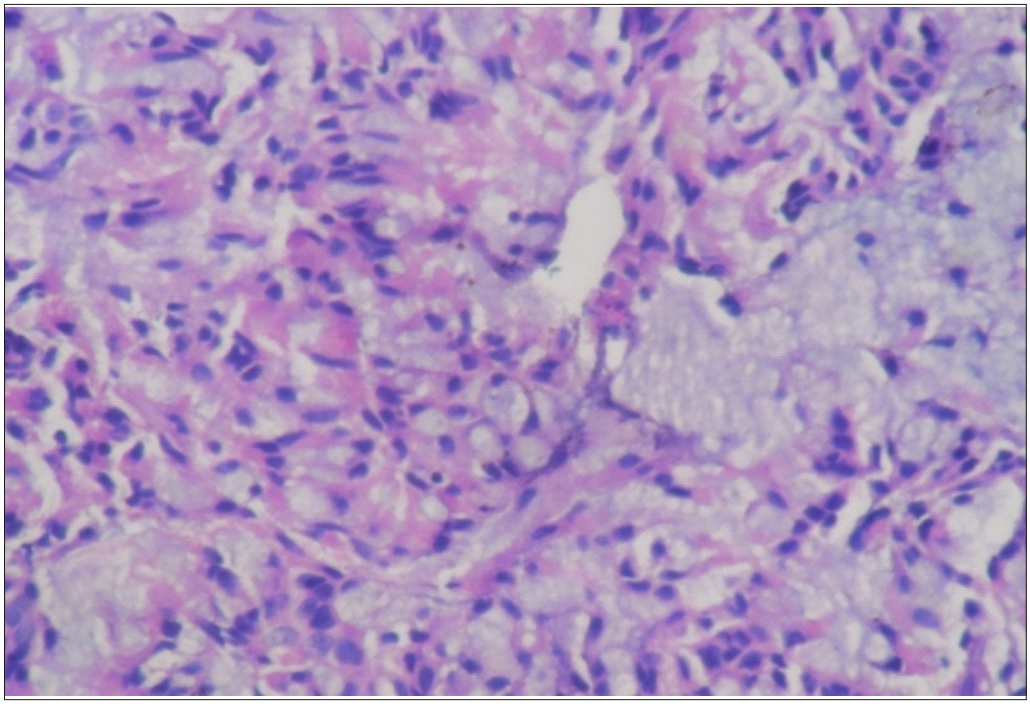


FIGURE:18 Adenocarcinoma –Mucinous type
100X HPE NO:7491/13

ALCIAN BLUE/PAS -POSITIVE

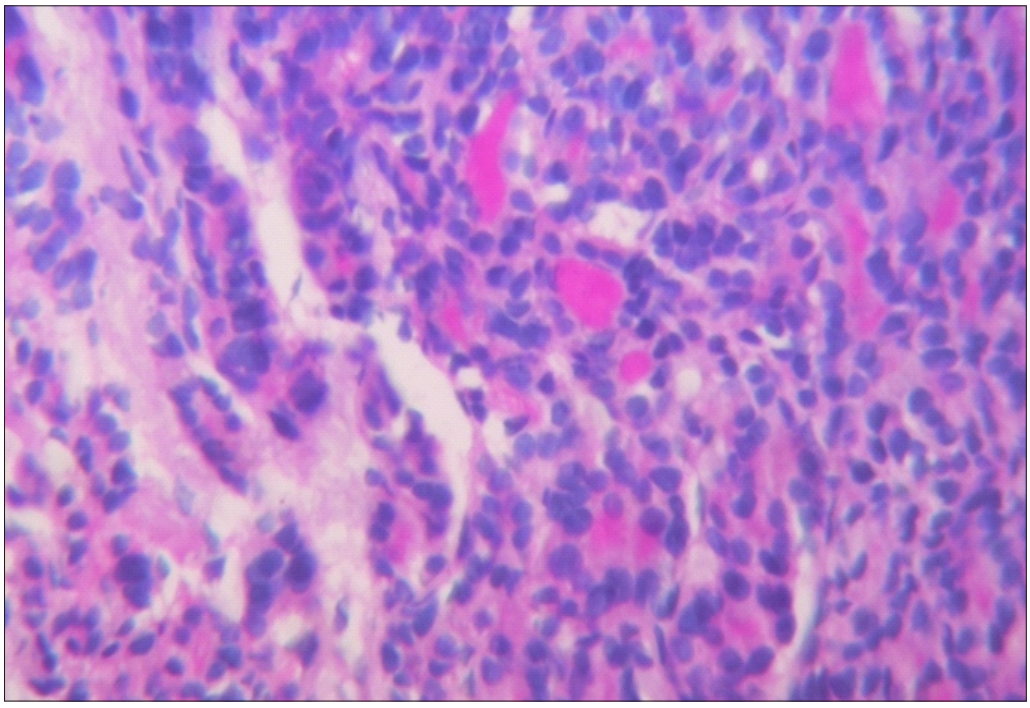


FIGURE:19 Neutral mucin positive adenocarcinoma
400X HPE NO:11378/13

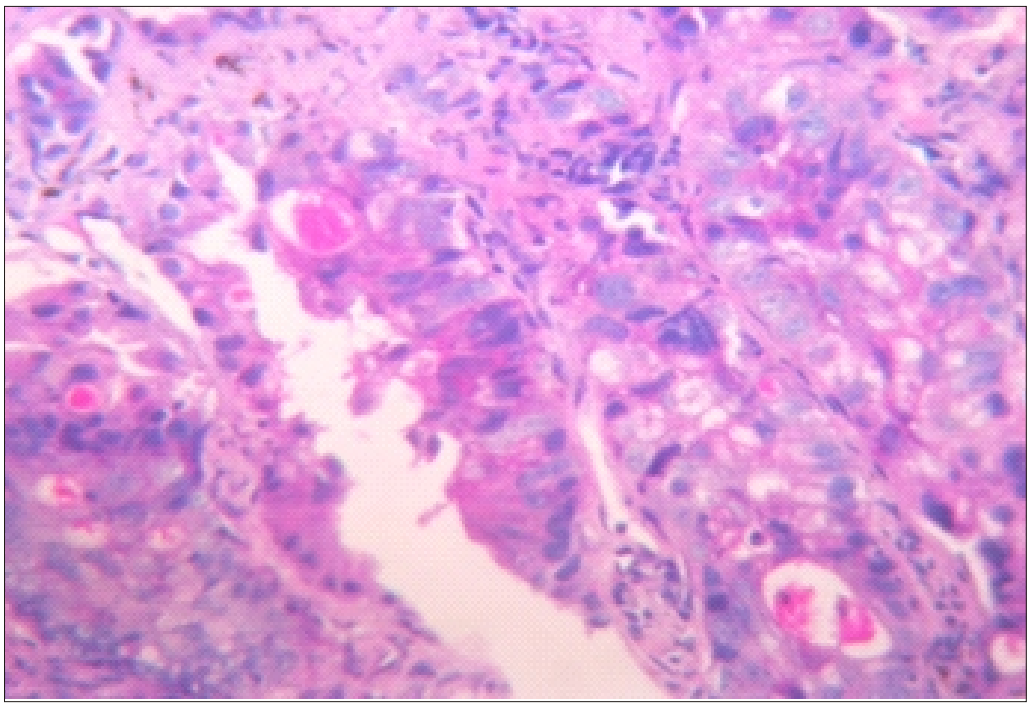


FIGURE:20 Neutral mucin positive adenocarcinoma
400X HPE NO:701/14

TTF-1 EXPRESSION

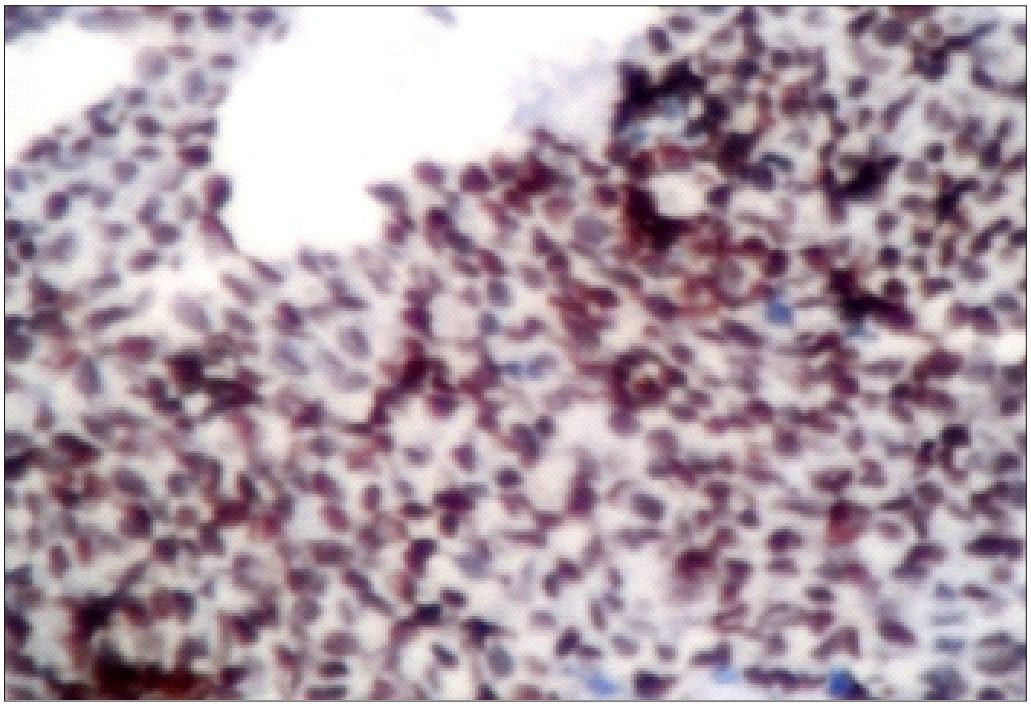


FIGURE:21 Strong nuclear positivity of TTF-1 in favoring adenocarcinoma
400 X HPE NO:2400/14

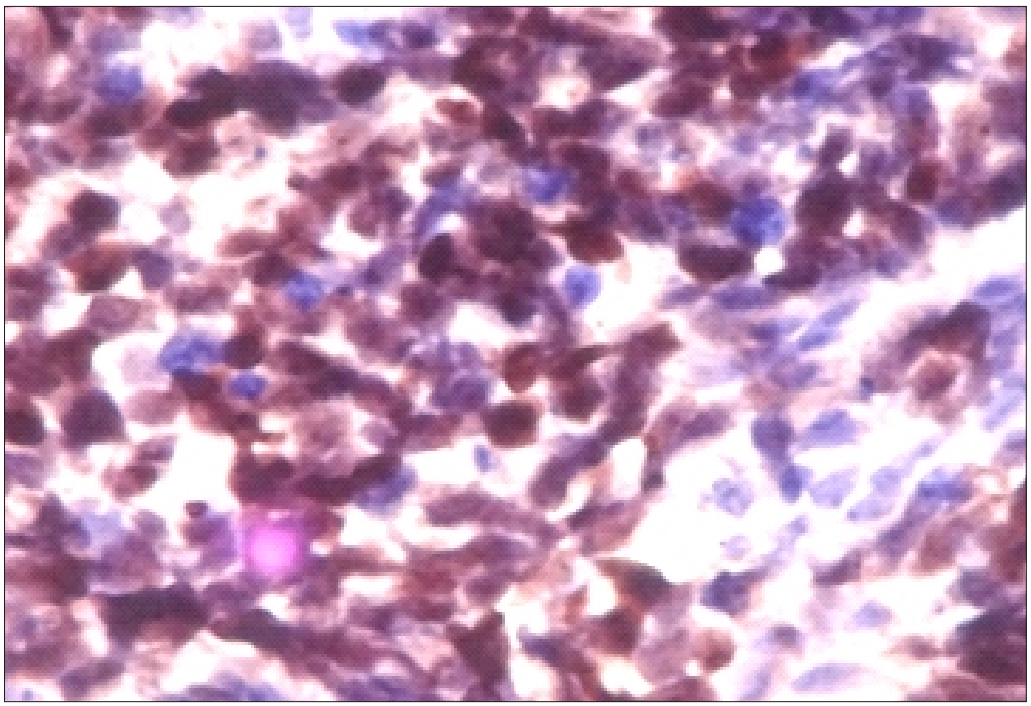


FIGURE:22 strong nuclear positivity in poorly differentiated adenocarcinoma
400X HPE NO:6167/14

p40 EXPRESSION

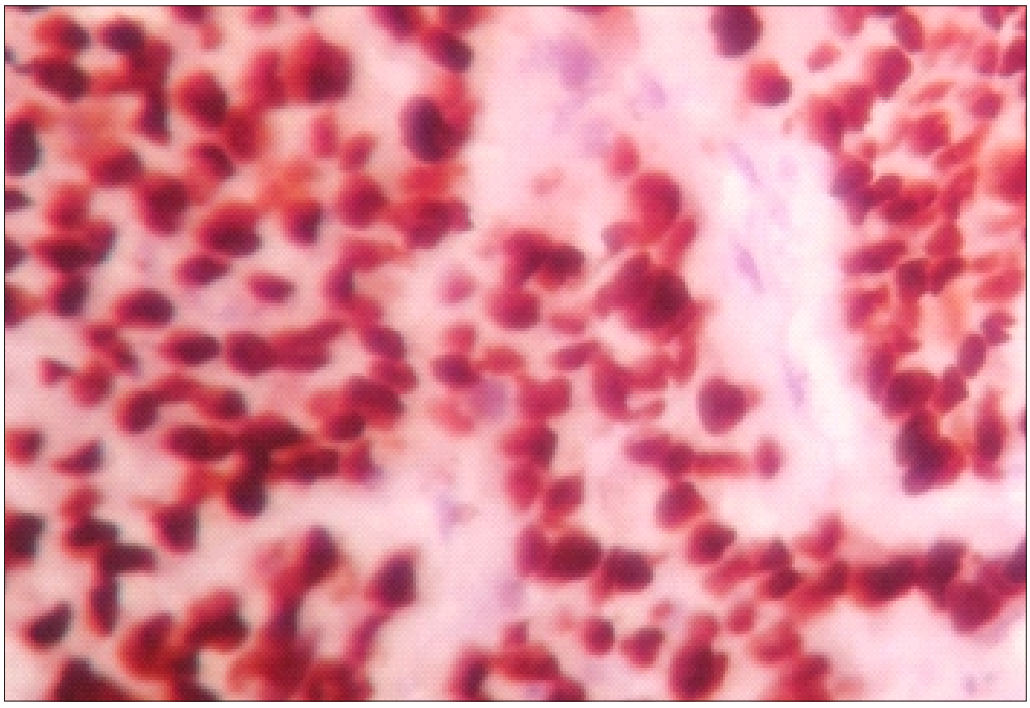


FIGURE:23 Strong nuclear positivity in poorly differentiated Squamous cell carcinoma 400X HPE NO:644/14

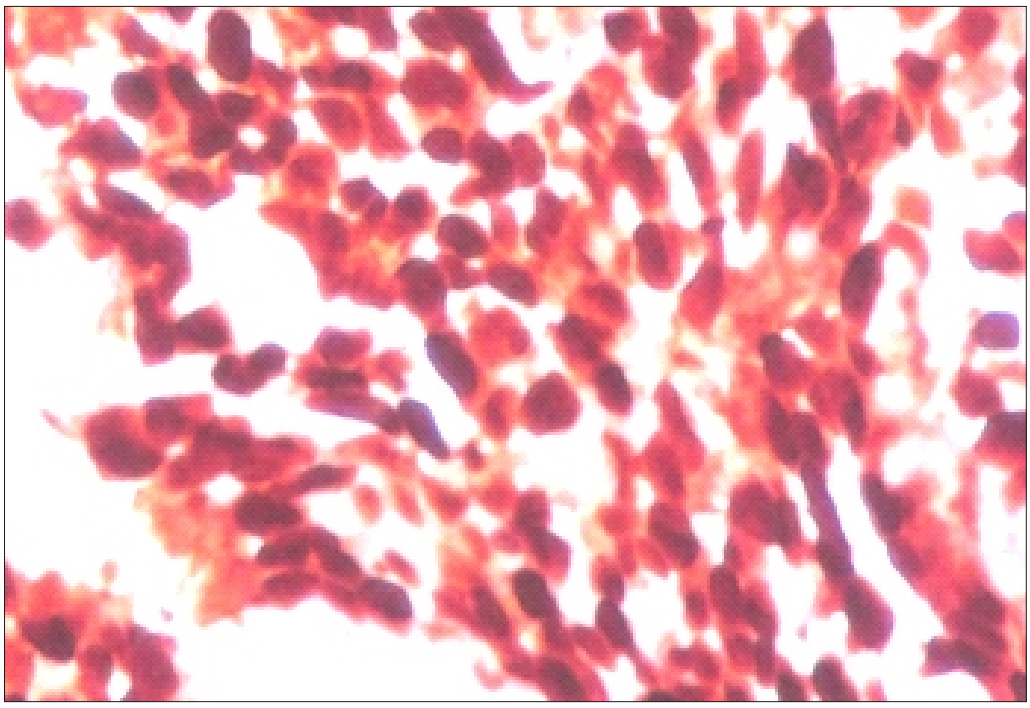


FIGURE:24 strong nuclear positivity in favouring squamous cell carcinoma 400 X HPE NO:5997/14

Discussion

DISCUSSION

Lung cancer is the leading cause of cancer and cancer related mortality around the world and most of the chemotherapeutic drugs used today lack adequate efficacy and specificity.

In India ,lung cancer has prepondarance to males constituting 10.9% all cases of cancer and 13% of all cancer related mortality. Mostly lung cancer affects people in 4th to 6th decade. Even with the recent advances available in treatment, the prognosis of the lung cancer still remains very poor.

85% of all lung cancers are non small cell lung carcinomas and adenocarcinoma is the commonest histologic type seen. Until recently, the histological subclassification of NSCLCs had no clinically or therapeutic value[(149).] But now with the availability of targeted therapy and its differential activity and adverse effects based on the type of the tumour, precise sub typing of non small cell lung carcinoma has become mandatory as it has direct impact on treatment and prognosis.

The NSCLC has to be further subclassified into more accurate subtypes on small biopsy such as squamous cell carcinoma and adenocarcinoma , whereever possible as it serves several purposes like

1. EGFR mutations should be tested in adenocarcinoma ,adenosquamous carcinoma and NSCLC-NOS .Because EGFR TK inhibitors is the primary treatment available for adenocarcinoma of lung.[150]

2. Those with adenocarcinoma histology or NSCLC-NOS showed better outcome with pemetrexed . Gemcitabine has shown improved outcome in patients with SCC.[151,152]

3. When Bevacizumab is used in patients with SCC of lung, life threatening haemorrhage can occur.[153]

Most of lung cancers diagnosed presents in advanced stage and resection rates were very low ,possible only in10 to 15 % of cases. So these few cases only will undergo complete histological examination. currently as the 2004 WHO classification for lung cancer, is based on resected specimens, the application of this classification has limited value. But now with more therapeutic options other than resections available, the diagnosis based on small biopsy and cytology became the primary method.

On comparison with The current WHO classification of lung cancer , the newer classification provided by IASLC/ATS/ERS 2011 has included the criteria for diagnosing lung cancer in small biopsy and cytology.

This is a retrospective and prospective study conducted between july 2013 to june 2014 in which 151 cases of non small cell lung cancer diagnosed at our institute were selected. On classifying them according to 2004 WHO classification, 121 cases were morphologically subtyped and 30 cases were classified as NSCLC-NOS.These NOS specified cases were further sub classified using Special stains (ALCIAN BLUE/PAS) and tumour markers (TTF-1 for adenocarcinoma and p40 for SCC). These special stains and markers are also used in selected cases of proven poorly differentiated Adeno and squamous cell carcinoma (10 cases each of ADC and SCC of lung) to test their efficiency in diagnosing NSCLCs. Based on these all the cases are classified according to the new IASLC/ATS/ERS international classification for the lung cancer. These results were compared with 2004 WHO classification of lung cancer.

Madras medical college is a tertiary referral center and in the institute of pathology ,about 1.44% were reported as lung cancer among the specimens received during the period of july 2013 to june 2014. Among the entire lung specimens received for histopathological examinations, 48.21% of the cases were reported to be malignant. Among the malignant cases, NSCLC accounts for 80.95% and SCLC accounts for 6.8%. This correlates with study done by **Navada S et al**[154], according to which most common type of lung cancer is NSCLC and it accounts for 80-85%.

This study showed that SCC of lung are the most common subtype which constitutes 51.65% followed by adenocarcinoma (29.1%) . This correlates with study done by **Delik Erman et al**[155] which says 60% of cases were squamous cell carcinoma and 30% were adenocarcinoma.

Squamous cell carcinoma:

In this study squamous cell carcinoma entity constitutes the predominant type of non small cell lung carcinoma .[155]

Squamous cell carcinoma was diagnosed histologically based on the presence of intercellular bridges and keratin formation. In the absence of these features ,it can be diagnosed on small biopsy based on the features like intraepithelial in- situ like extension along the bronchus. These features are not seen in other carcinomas such as small cell or adenocarcinoma. **Suprun H et al**[33]. In our study ,all the cases of squamous cell carcinoma showed intercellular bridges and keratinisation.

Among the 151 cases, 78 cases (41.9%) come under this category and showed male predominance with 66 cases(84.4%) with male to female ratio was 5.6:1. The predominant age group of involvement was 51to 60 years, in which 23 cases(29.48% with the mean age of 57.96 years) were reported . One case was reported under the age of 30 years. 7 (8.89%) cases were reported above 70 years.

This finding correlates with study done by **Delik Erman et al** [155], according to which NSCLCS has predilection in the age group of 50-80 years. Only 3% of cases presented before 30 years.

Cough ,breathlessness and weight loss were the most common clinical presentation of squamous cell carcinoma. According to **Bach PB et al**[156], common symptoms of lung carcinomas are persistent cough, hemoptysis, chest pain, shortness of breath , hoarseness and repeated respiratory infections, e.g. bronchitis, pneumonia.

Out of the total 78 cases of squamous cell carcinoma, 55 cases(70.5%) were associated with history of smoking. This was correlated with study done by **Satcher D et al**[157], who found that most common cause of lung cancer in both men and women is was smoking and had strongest association with smoking was seen with SCC.

Most of the SCC were found in the right upper lobe constituting 34.61%%(n=27) followed by left upper lobe constituting 26.92% (n=21) of cases. This showed that the prevalence of squamous cell carcinoma were seen in the upper lobes. This correlates with the study done by **Davies DF et al**[158]

Most common radiographic finding of squamous cell carcinomas was found to be central or hilar mass lesion.This correlates with the study done by **Minna, JD et al**.[159]

According to **Sharma SK et al**[160], growth, necrotic material and inflamed mucosa were the most common findings in fibroptic bronchoscopy. Similarly in this study also with the available FOB findings, most of the squamous cell carcinoma was presented with endobronchial growth and other findings are inflamed mucosa and narrowed bronchus.All the polypoidal lesion showed squamous cell carcinoma histology.

Among the 26 bronchial wash cytology ,11 cases of SCC diagnosed were correlated with the 42.30%of biopsies. Most of sputum cytology were positive for squamous cell carcinomas.

Among 78 cases of squamous cell carcinoma, local metastasis were seen in 11 cases (14.10 %), 9 cases(11.53%) showed regional metastasis and 8 cases (10.25%) showed distant metastasis .

ADENOCARCINOMA:

In this study, adenocarcinoma constitutes the second most common type of NSCLC next to SCC . The diagnosis of adenocarcinoma was made on the basis of glandular structure and mucin production.

Among the total cases o f 151 cases, 29.1% (n=44) cases comes under this category. The predominant age group was 51 to 60 years with the mean age of 57.02 years.15cases were noted in this category with the percentage of 34%. No cases were reported under the age of 30 years. Out of 44 cases ,59%(n=26) were males and 40.9% (n=18) were females with the male to female ratio of 1.44:1.

Out of 44 cases of adenocarcinoma 36.36% of cases were associated with history of smoking.Several studies concluded that the most common cause of lung cancer in both men and women was smoking of tobacco products.

Bain et al[161]found that the incidence of lung cancer among the non smokers,more common in men than women.This observation was in concurrence with this study. American cancer society have reported that currently the incidence of lung cancer in females is nearly equal to that of males.[162] Out of these 44 cases of adenocarcinoma 36.36% of cases were associated with history of smoking. Several studies concluded that the most commom cause of lung cancer in both men and women are due to smoking of tobacco products .

Most of the adenocarcinoma were seen in right upper lobe 25%(n=11) of cases, followed by left upper lobe constitutes 18.18% (n= 8) cases.[159]

Right lung was most commonly involved than the left which is in concurrence with the study done by **Vivekanand N et al** in which cancers of right lung (56.6%) is more common than the cancers of left lung (43.30%) in a ratio of 1.3:1,respectively. Most common radiologic finding of adenocarcinoma was found to be peripheral mass and opacity.[160]

Among these 44 cases of adenocarcinoma, 9 cases(20.45%) were found with local metastasis, 5cases(11.36%) with regional metastasis and 10 cases (22.72%) with distant metastasis. in this study ,the metastasis were found to be more common with adeno carcinoma than SCC. This is inconcurrence with the study done by **collins LG et al** and **Alberg AJ et al**.[163,164]

With fibroptic bronchoscopic findings most of the adenocarcinomas are presented with endobronchial growth. Conflictingly few cases which were normal /inflammed mucosa on FOB are reported as malignancy. It is comparable with to study by **Schreiber G et al** which says few of the lesions had no evidence of growth (or) mucosal abnormalities by FOB were reported as malignant.

Mixed subtype(38.6%) of ADC when classified based on WHO2004 classification outnumbered other subtypes in the order of solid type (25%), acinar type (18.8%), papillary and bronchoalveolar subtypes each constituting 9%. though its the predomonant type the percentage reported was lower

compared to the study done by **Akihiko yoshizawa et al**[165], where it found 95 % of the patients classified into mixed group.

When adenocarcinomas of this study were subtyped based on patterns in accordance with the new IASLC/ATS/ERS classification , solid type (30.50%) predominates followed by acinar type (20.33%), papillary type (10.16%), lepidic pattern (8.4%) and micropapillary pattern(3.38%) .

In this study, Major differences identified between WHO and IASLC classification includes ,

One case of large cell carcinoma reported according to WHO classification was termed as NSCLC -NOS in the newer classification as the diagnosis of large cell carcinoma can be possible only with resected specimens. large cell carcinoma diagnosis is possible only with resected specimens as it is a diagnosis by exclusion **Scagliotti G et al**[175] **and Travis WD et al.**[113]

One case of sarcomatoid carcinoma diagnosed based on WHO classification is classified under NSCLC-NOS in the new classification as diagnosis of sarcomatoid carcinoma cannot be made in small biopsies because there is difficulty in assessing the mixed patterns. Diagnosis of sarcomatoid carcinoma is made by presence of atleast 10% of giant and/or spindle cells and it is possible to assess only in resected specimens.

With 38.6% of the cases were subclassified into mixed subtype ,the 2004 WHO classification has not provide any prognostic significance. [165,166].

According to IASLC classification , the mixed type was further subtyped based on the presence of predominant pattern as acinar (46.15%), papillary (19.23%), solid (23.07%), lepidic (3.84%) and micropapillary(7.69%). Among these solid and micropapillary type indicates poor prognosis. Thus new classification on contrast to WHO 2004 classification helps to prognosticate the specific subtype.

According to WHO classification Bronchoalveolar carcinoma constitutes 9% of adenocarcinomas. The term bronchoalveolar carcinoma is used for spectrum of adeno carcinoma tumours in resected specimens which have 100% to 15-20% of 5 year survival rate. But new IASLC classification does not include the term bronchoalveolar carcinoma. Instead it classifies previous Non- Mucinous BAC as Adenocarcinoma with lepidic pattern which has favourable outcome (in this study it constitutes about 11.62% of cases) and Mucinous BAC as mucinous adenocarcinoma which has poor prognosis.

In this study micropapillary adenocarcinoma constitutes 4.65% ,which is not included in WHO classification but included in IASLC classification which have poor prognosis.

Kazunori et al[167], observed that the micropapillary pattern is a distinct variant in lung adenocarcinoma with prognostic significance. On pathological diagnosis this variant should be diagnosed carefully. Prognosis is poor even in very early stage for the micropapillary variant . **Miyoshi T,Satoh Y et al**[168], also found that the presence of micropapillary pattern have a prognostic value . In addition it was observed that micropapillary pattern may be associated with invasion into lymphatic system and nodal metastasis and the expression of surfactant apoprotein A in a micropapillary area attributes to poor prognosis in small size adenocarcinoma of lung.

Several studies have demonstrated the prognostic significance of new IASLC/ATS/ERS classification.

Morphologic diagnosis always forms the basis of diagnosis and is further supplemented by immuno histochemical markers. In this study, the panel of AB/PAS, TTF-1 and p40 were used in 50 cases of poorly differentiated NSCLCs. Alcian blue-PAS positive in (n=20) 40% of cases.TTF-1 was positive in (n=25) 50% of cases and p40 was positive in(n=21) 42% 0f cases. Both TTF-1 and p40 were negative in (n=3) 6% of cases.

In this study , among the 10 cases classified by WHO as poorly differentiated adenocarcinoma , AB/PAS showed positivity in 70% of cases

and TTF-1 showed positivity in 80% of cases. Sensitivity of TTF-1 was found to be 100%,and specificity was found to be 91%.

According to **Kennedy et al**[,(169)] Mucin stains are valuable markers, but it shows variable sensitivity and specificity for adenocarcinoma. It is one classical characteristic feature of pulmonary adenocarcinoma ,but still it is not seen in all cases . In addition ,study by **Kennedy et al,**[(169)] found that by the use of PAS staining method the percentage of positive (90%) is slightly less when compared to the higher percentage of positivity (93%) obtained by the combined Alcian blue –PAS method. Hence the combination staining method using Alcian blue –PAS seems to give more satisfactory results.

In this study the TTF-1 positivity were noted in 96.15% of adenocarcinoma. According to the study by **Stenhouse G et al**[(170),] TTF-1 as a marker for adenocarcinoma was found to have specificity of 97-100%, but the sensitivity rate was low at 54-75%. It also showed that in subtyping of tumours in the undifferentiated NSCLC category they found that the expression of TTF1 marker was present in 70 to 85% of lung adenocarcinomas . On an average the positivity for TTF1 marker in morphologically diagnosable adenocarcinomas ranges from 60 to 92%.

Kaufmann et al[(171),]concluded that 75% of nonmucinous adenocarcinomas demonstrated TTF-1 positivity. whereas mucinous

adenocarcinoma showed positivity only in 10%. Therefore the combined usage of TTF-1 and mucin stain AB/PAS has more significant value in the diagnostic panel.

In our study one case of poorly differentiated adenocarcinoma was negative for TTF-1 stain , but, on the other hand it showed positivity for AB/PAS stain . Hence in correlation with **Kauffman et al [171],** owing to the positivity of mucin stain in this study it is diagnosed as fovouring adenocarcinoma

In the present study p40 showed positivity in all the 10 cases classified by WHO as poorly differentiated squamous cell carcinoma.

According to **Wei zhao et al.,** [172]p40 is considered to be the best marker in differentiating SCC and adenocarcinoma. p40 has sensitivity of 100%,and specificity of 100%. Present study is in accordance with this view. In the recent study by **Bishop et al** , in terms of sensitivity for SCC ,it was found to be equal for the two markers p40 and p63, but in terms of specificity p40 proved to be superior. Though p63 is the marker for SCC it can show positivity in some cases of adenocarcinoma or an unsuspected lymphoma. so p40 is the ideal marker in the diagnosis of SCC of lung. Another study by **Pelosi G et al**, also strongly recommend p40 as a reliable and more superior marker for SCC.

Hence the use of p40 in conjunction with TTF-1 was justified to be a promising diagnostic tool in the routine practise for subtyping of NSCLC.

Giuseppe Pelosi et al[173], also emphasized the application of two marker panel which comprises of p40 and TTF-1 on small biopsy or cell block samples was considered more effective and reliable in correctly subtyping a vast majority of lung cancers. Similar findings were recorded in yet another study by "**Brown and colleagues et al''**[174] who concluded that the use of TTF-1/p40 cocktail on small biopsy for differentiating lung SCC and ADC was very effective. The added benefit is that we can employ this cocktail on a single slide.

In this study, among the 151 cases of NSCLCs, 30 cases belonged to not otherwise specified (NOS) constituting 19.86% of the total. But with the use of Mucin stains, and IHC markers TTF-1 and p40 , it was minimised to 1.98%

Also in this study, when intrepreted according to the new classififcation, among these 30 cases of undifferentiated non small cell carcinoma (NOS) group, were subtyped using special stains and IHC markers into

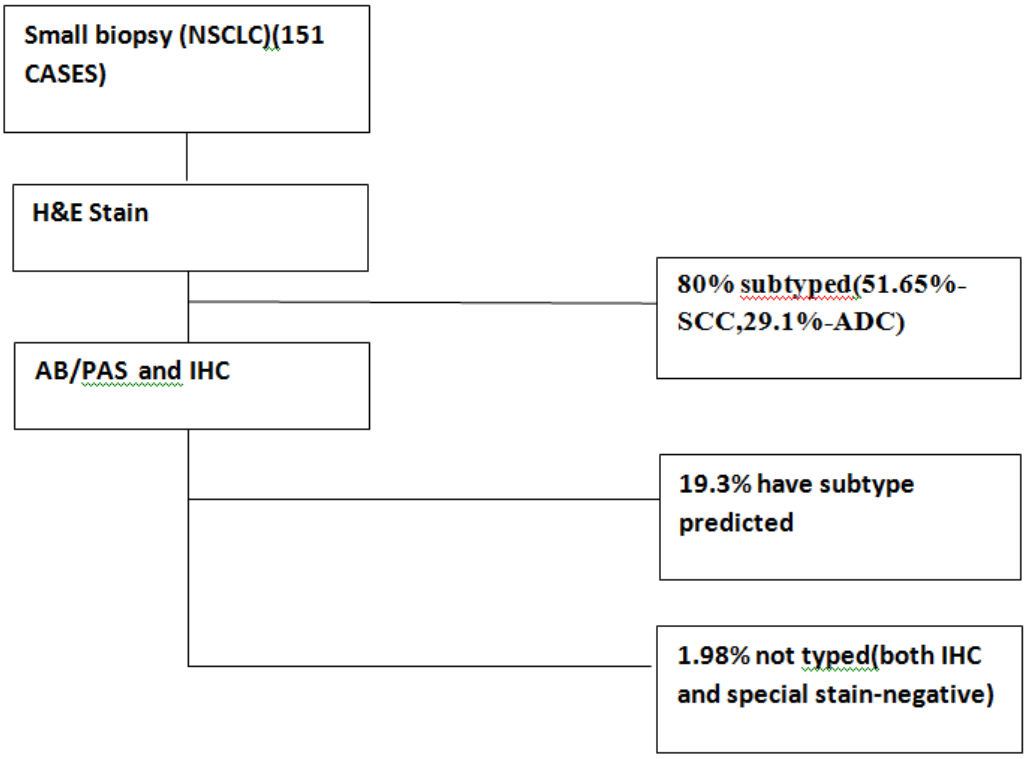
Favouring adenocarcinoma 15 cases (50 %),

Favouring squamous cell carcinoma 11cases (36.66%),

Possible adenosquamous carcinoma was 1 case(3.33%)and

Unsubtyped were 10%(n=3).

Diagnostic algorithm for classification on NSCLC in small biopsy:



Summary

SUMMARY

- Among the total number of 392 lung specimens received in the institute of pathology ,Madras Medical College during the study period of july 2013 to june 2014, 323 were small biopsy specimens and among them 183 were reported as malignancy with relative percentage of 56.65%.

- Non small cell lung carcinoma (82.51%) vastly outnumbered the small cell lung carcinoma (8.60%) in our study.

- The distribution of SCC and ADC was 51.65% and 29.1% respectively. Thus squamous cell carcinomas are more frequent than adenocarcinoma. NSCLC-NOS accounted for 19.8%.

- Among the 50 cases of poorly differentiated NSCLCs, Alcian blue-PAS was positive in 40% of cases. TTF-1 was positive in 50% of cases and p40 was positive in 42% of cases. Both TTF-1 and p40 were positive in 2% of the cases. Both TTF-1 and p40 were negative in 6% of cases.

- One case of poorly differentiated adenocarcinoma was negative for TTF-1 stain , but on the other hand, it showed positivity for AB/PAS stain . Owing to the positivity of mucin stain in this case it was diagnosed as NSCLC favouring adenocarcinoma.

- Undifferentiated group not otherwise specified constituting 19.86% of the total of 151 cases. But with the use of Mucin stain, and IHC markers TTF-1 and p40 , it was minimised to 3%.

- According to IASLC s new classififcation, undifferentiated non small cell carcinoma (NOS) group, were subtyped using special stains and IHC markers into favouring adenocarcinoma 50%, favouring squamous cell carcinoma 36.66%,possible adenosquamous carcinoma 3.33% and unsubtyped were 10%

- 38.6% of cases of Adenocarcinoma were subclassified into mixed subtype , based on 2004 WHO classification

- According to IASLC classification , the mixed type was further subtyped based on the presence of pattern as acinar (46.15%), papillary (19.23%), solid (23.07%), lepidic (3.84%) and micropapillary(7.69%). Among these solid and micropapillary type indicates poor prognosis. Thus new classificationof IASC, in contrast to WHO 2004 classification helps to prognosticate the specific subtype.

- According to WHO classification Bronchoalveolar carcinoma constitutes 9% of adenocarcinomas. But the term bronchoalveolar

carcinoma is no longer used. As per IASC classification, it is termed as Adenocarcinoma with lepidic pattern.

- In this study micropapillary adenocarcinoma constitutes 4.65% ,which is not included in WHO classification but included in IASLC classification which have poor prognosis.

Conclusion

CONCLUSION

- International classification for lung cancer needs a special mention is that it is the first ever engineered classification for lung malignancies in small biopsy /cytology.

- According to this study we conclude that multidisciplinary International Classification For Lung Cancer is superior to 2004 WHO classification in terms of diagnostic, therapeutic and prognostic implications.

- As a surgical pathologist ,our role does not end in classifying lung malignancies as SCLC and NSCLC but has to be subtyped using special stains and IHC markers. Alcian blue-PAS and IHC markers like TTF-1 and P40 are found to be extremely efficient and useful markers.

- Furthermore ,in this era of targeted therapy, basic histopathological diagnosis per se is not enough but further histological subtyping as well as molecular testing are of paramount importance, as these are the key factors determining the treatment options.

- Thus the surgical pathologist can be considered as the sole guardian of these small biopsy specimens-The very precious yet limited resource from which one needs to maximise the diagnostic yield.

Annexures

ANNEXURE-I

PROFORMA

Case number : Name :

HPE number : Age :

IP number : Sex :

Clinical diagnosis :

Complaint :

Radioimaging :

FOB findings :

Site of lesion : Right upper lobe/middle lobe/lower lobe

Left upper lobe/hilum/lower lobe

Specimen :CT guided biopsy/Bronchial biopsy/USG guided biopsy/Open biopsy

MICROSCOPY : Histological diagnosis according to WHO classification

Special stain :

Alcian blue/PAS

IHC

TTF-1 : Positive / Negative

p40 : Positive / Negative

DIAGNOSIS :According to IASLC/ATS/ERS classification

ANNEXURE:II
WHO CLASSIFICATION FOR LUNG CANCER

Malignant epithelial tumours

Squamous cell carcinoma
- Papillary
- Clear cell
- Small cell
- Basaloid

Small cell carcinoma
- Combined small cell carcinoma

Adenocarcinoma
- Adenocarcinoma, mixed subtype
- Acinar adenocarcinoma
- Papillary adenocarcinoma
- Bronchioloalveolar carcinoma
  - Nonmucinous
  - Mucinous
  - Mixed nonmucinous and mucinous or indeterminate Solid adenocarcinoma with mucin production
- Fetal adenocarcinoma
- Mucinous ("colloid") carcinoma
- Mucinous cystadenocarcinoma
- Signet ring adenocarcinoma
- Clear cell adenocarcinoma

Large cell carcinoma
- Large cell neuroendocrine carcinoma
  - Combined large cell neuroendocrine carcinoma
- Basaloid carcinoma
- Lymphoepithelioma-like carcinoma
- Clear cell carcinoma
- Large cell carcinoma with rhabdoid phenotype

Adenosquamous carcinoma

Sarcomatoid carcinoma
- Pleomorphic carcinoma
- Spindle cell carcinoma
- Giant cell carcinoma
- Carcinosarcoma
- Pulmonary blastoma

Carcinoid tumour
- Typical carcinoid
- Atypical carcinoid

Salivary gland tumours
- Mucoepidermoid carcinoma
- Adenoid cystic carcinoma
- Epithelial-myoepithelial carcinoma

Preinvasive lesions
- Squamous carcinoma in situ
- Atypical adenomatous hyperplasia
- Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia

Mesenchymal tumours
- Epithelioid haemangioendothelioma
- Angiosarcoma
- Pleuropulmonary blastoma
- Chondroma
- Congenial peribronchial myofibroblastic tumour
- Diffuse pulmonary lymphangiomatosis
- Inflammatory myofibroblastic tumour
- Lymphangioleiomyomatosis
- Synovial sarcoma
  - Monophasic
  - Biphasic
- Pulmonary artery sarcoma
- Pulmonary vein sarcoma

Benign epithelial tumours

Papillomas
- Squamous cell papilloma
  - Exophytic
  - Inverted
- Glandular papilloma
- Mixed squamous cell and glandular papilloma

Adenomas
- Alveolar adenoma
- Papillary adenoma
- Adenomas of the salivary gland type
  - Mucous gland adenoma
  - Pleomorphic adenoma
  - Others
- Mucinous cystadenoma

Lymphoproliferative tumours
- Marginal zone B-cell lymphoma of the MALT type
- Diffuse large B-cell lymphoma
- Lymphomatoid granulomatosis
- Langerhans cell histiocytosis

Miscellaneous tumours
- Harmatoma
- Sclerosing hemangioma
- Clear cell tumour
- Germ cell tumours
  - Teratoma, mature
  - Immature
  - Other germ cell tumours
- Intrapulmonary thymoma
- Melanoma

Metastatic tumours

ANNEXURE:III

PROPOSED IASLC/ATS/ERS CLASSIFICATION FOR SMALL BIOPSIES/CYTOLOGY

2004 WHO Classification	**SMALL BIOPSY/CYTOLOGY: IASLC/ATS/ERS**
ADENOCARCINOMA Mixed subtype Acinar Papillary Solid	Morphologic Adenocarcinoma pattern clearly presents:Adenocarcinoma, describe identifiable patterns present (including micropapillary pattern not included in 2004 WHO classification)
No 2004 WHO counterpart – most will be solid adenocarcinomas	Morphologic adenocarcinoma patterns not present (supported by special stains): Non-small cell carcinoma, favor adenocarcinoma
Bronchioloalveolar carcinoma (nonmucinous)	Adenocarcinoma with lepidic pattern (if pure, add note: an invasive component cannot be excluded)
Bronchioloalveolar carcinoma (mucinous)	Mucinous adenocarcinoma (describe patterns present)
Fetal	Adenocarcinoma with Fetal pattern
Mucinous (colloid)	Adenocarcinoma with colloidal pattern
Signet Ring	Adenocarcinoma with (describe patterns present) and signet ring features
Clear cell	Adenocarcinoma with (describe patterns present) and clear cell features
SQUAMOUS CELL CARCINOMA Papillary Clear cell Small cell Basaloid	Morphologic squamous cell pattern clearly presents: Squamous cell carcinoma
No 2004 WHO counterpart	Morphologic squamous cell patterns not present (supported by stains): Non-small cell carcinoma, favor squamous cell carcinoma
SMALL CELL CARCINOMA	Small cell carcinoma
LARGE CELL CARCINOMA	Non-small cell carcinoma, not otherwise specified (NOS)

Large cell neuroendocrine carcinoma (LCNEC)	Non-small cell carcinoma with neuroendocrine (NE) morphology (positive NE markers), possible LCNE
Large cell carcinoma with N morphology(LCNEM)	Non-small cell carcinoma with NE morphology (negative NE markers) – see comment Comment: This is a non-small cell carcinoma where LCNEC is suspected, but stains failed to demonstrate NE differentiation.
ADENOSQUAMOUS CARCINOMA	Morphologic squamous cell and adenocarcinoma patterns present: Non-small cell carcinoma, NOS, (comment that glandular and squamous components are present
No counterpart in 2004 WHO classification	Morphologic squamous cell or adenocarcinoma patterns present and stains are conflicting (TTF1 and p63 positive) or suggest the other pattern is also present Non-small cell carcinoma, NOS, comment that glandular and squamous differentiation seen by IHC) Comment (for either setting): this could represent adenosquamous carcinoma.
Sarcomatoid carcinoma	Poorly differentiated NSCLC with spindle and/or giant cell carcinoma (mention if adenocarcinoma or squamous carcinoma are present)

ANNEXURE:IV

IASLC/ATS/ERS CLASSIFICATION OF LUNG ADENOCARCINOMA IN RESECTION SPECIMENS

PREINVASIVE LESIONS

A typical adenomatous hyperplasia

Adenocarcinoma in situ (≤3 cm formerly BAC)

-Nonmucinous

-Mucinous

-Mixed Mucinous/Non-Mucinous

MINIMALLY INVASIVE ADENOCARCINOMA (≤3 cm lepidic predominant tumor with ≤5 mm invasion)

-Non mucinous

-Mucinous

-Mixed Mucinous/Non-Mucinous

INVASIVE ADENOCARCINOMA

Lepidic predominant (formerly non-mucinous BAC pattern, with >5 mm invasion)

Acinar predominant

Papillary predominant

Micropapillary predominant

Solid predominant with mucin production

VARIANTS OF INVASIVE ADENOCARCINOMA

Invasive mucinous adenocarcinoma (formerly mucinous BAC)

Colloid Fetal (low and high grade)

Enteric

ANNEXURE-V

Special staining (PAS/ALCIAN BLUE) procedure:

1. Bring sections to distilled water.
2. Stain with Alcian blue 15 mins.
3. Wash well in running tap water for 2 mins.
4. Rinse in distilled water.
5. Treat with periodic acid 5 mins.
6. Wash well in distilled water.
7. Stain with Schiff's reagent for 10 mins.
8. Wash well in running tap water for 5 mins.
9. Stain nuclei with haematoxylin for 1 min.
10. Wash in running tap water for 2 mins.
11. Differentiate with acid alcohol.
12. Wash and blue nuclei in Scott’s tap water.
13. Wash in water.
14. Dehydrate, clear and mount.

Results will be interpreted as

- Neutral mucins will appear in Magenta
- Acidic mucins in Blue
- Nuclei in Deep blue
- Mixtures of above in Blue/purple

ANNEXURE-VI

Immunohistochemistry procedure:

Slide Preparation:

1. Sections with a thickness of 4 µ were cut from formalin fixed paraffin embedded tissue samples and transferred to gelatin-chrome alum coated slides.
2. The slides were incubated for overnight at 58ºC.
3. The sections were deparaffinised in xylene for 15 minutes x 2 changes.
4. The sections were dehydrated with absolute alcohol for 5 minutes for 2 changes.
5. Then the sections were washed with tap water for 10 minutes.
6. The slides are then immersed in distilled water upto 5 minutes.

Antigen Retrieval:

1. Heat induced antigen retrieval was done with microwave oven in appropriate temperature with appropriate buffer for 20 minutes. This step unmasks the antigenic determinants of fixed tissue sections.
2. The slides were then cooled to room temperature for 20 minutes and washed with tap water for 5 minutes.
3. The slides were then rinsed with distilled water for 5 minutes.
4. then the slides were washed with appropriate wash buffer (phosphate buffer) for 5 minutes x 2 changes.
5. Peroxidase block was then applied for 10 minutes.
6. The slides then were washed in phosphate buffer for 5 minutes x 2 changes.
7. Sections were covered with protein block for 5 minutes.

Antibody application:

1. The sections were drained (without washing) and appropriate primary antibody is applied and incubated for 30 minutes.
2. The slides were washed in the phosphate buffer for 5 minutes x 2 changes.
3. The slides were covered with Primary antibody amplifier for 10 minutes.
4. The slides were washed in the phosphate buffer for 5 minutes x 2 changes.
5. The slides were covered with HRP micropolymer Quanto for 10 minutes.
6. The slides were washed in the phosphate buffer for 5 minutes x 2 changes.

Chromogen application:

1. DAB substrate was prepared by diluting 1 drop of DAB Quanto chromogen to 1 ml of DAB Quanto buffer.
2. DAB substrate solution was applied on the sections for 5 minutes.
3. wash the slides then in distilled water for 2 minutes.
4. counterstain the section with Hematoxylin for 2 seconds.
5. wash the slides in running tap water for 5 minutes.
6. air dry the slides, cleared with xylene and mounted with DPX.

Alternate methods of antigen retrieval

- Pressure cooker antigen retrieval
- Microwave and trypsin antigen retrieval

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MASTER CHART

HPE No.	Age	Sex	Site	Smoking	C/F	X-ray, CT, MRI	FOB	Cytology	Specimen	HPE Diagnosis	P40	TTF1	MUCIN	IASLC
60/14	52	M	4	Y	1,2,5	>3cm	M	LN-Mets	2	S				S
6/14	70	M	1	Y	1,3,5	M,ME	M	BW -pos	1	S	P	N	N	S
178/14	67	M	3	N	1,3,4,5	M,C,B/L	I M		2	N PD	P	N	N	F S
217/14	63	M	1	Y	1,5	>3cm,C,M	I M		1	S				S
462/14	55	M	1	Y	1,4,5	M,LI	P	BW -POS	2	S				S
476/14	83	M	4	Y	1,3,5	M,CN			1	S				S
644/14	65	M	4	Y	3,4,5	M,A	M	SP-POS	3	S	P	N	N	S
690/14	55	M	3	Q	1,3,5	M,MNI	M	SP-POS	2	S	P	N	N	S
701/14	75	M	2	N	1,3,5	MX N,MNI			1	A S	N	P	P	A S
1014/4	56	M	3	Y	1,5	<3cm,F		BW-POS	1	A S	N	P	P	A S
1054/14	62	M	5	Y	1,3,5	M,MNC,B/L,PE	M		2	N PD	P	N	N	F S
1565/14	65	M	2	N	1,2,5	O		SP-POS	1	S				S
1597/14	60	M	1	Y	1,3,5	<3cm	P	SP NEG	1	S PD	P	N	N	S
1630/14	56	M	3	N	1,3,4,5	O			1	N PD	N	P	P	F A
1744/14	55	M	3	N	1,3,4,5	M,MNI,C		SP-NEG	1	A,P				A,P
1830/14	55	M	1	Y	1,4,5	O	I M		2	N PD	N	P	N	F A
2031/14	45	F	5	N	4,5	<3cm			1	N PD	N	P	P	F A
2043/14	64	M	3	Y	1,2,4,5	>3cm			1	S				S
2120/14	48	M	4	Y	1,2	CON		PF NEG	1	S	P	N	N	S
2262/14	51	M	3	Y	1,2,5	>3cm			1	S	P	N	N	S
2293/14	57	M	2&5	N	1,3,4,5	M,MNPE	N B		1	A,M				A,A
2312/14	50	F	3	N	1,2,5	M,B	I M	PFNEG	1	S	P	N	N	S
2340/14	65	M	3	Y	1,4,5	M			1	N PD	P	N	N	F S
2344/14	63	M	6	Q	1,2,5	M,SCN	M	BW-NEG	1	S				S
2400/14	52	F	1	Y	3,4,5	M,CN			1	N PD	N	P	N	F A
2527/14	62	M	1	Y	1,2,5	>3cm	N		1	S				S
2521/14	75	M	3	Y	1,2,4,5	O	N		1	S				S
2531/14	56	F	1	N	1,3,4,5	>3cm	N		1	A S	N	N	P	A S
2532/14	56	F	3	N	1,2,5		M	BW-NEG	1	S				S
2618/14	61	M	1	Y	1,3,4,5	>3cm	NA		1	A,M				A,A
2644/14	70	M	5	N	1,2,5	H		BW-POS	1	S				S
2732/14	55	M	6	Y	4,5,3	M,B	N		1	N PD	P	N	N	F S
2881/14	60	M	1	Y	1,3,5	MXN			1	A M	N	P	P	A,A
3049/14	63	F	5	Y	1,2,3	>3cm	M		2	N PD	P	N	N	F S
3101/14	60	F	5	N	1,3,4	<3cm			1	A S	N	P	P	A S
3104/14	60	M	1	Y	1,2,5		M	SP-POS	2	S PD	P	N	N	S
3140/14	70	M	1	Y	1,2,5		M		2	S				S
3411/14	65	F	4	Y	1,5	CAV	M		2	S				S
3314/14	60	M	1	Y	1,4,5	M,Rib	M	BW-NEG	2	S				S
3836/14	58	M	1	N	1,2,5		M		2	S				S

3990/14	55	M	1	Y	1,4,5	>3cm,PE		BW-POS	5	S				S
4089/14	45	M	6	Y	1,3,5	H	M	BW NEG	3	A M				A,A
4308/13	72	M	4	N	1,3,5	M,ME	M		1	A M				A,MP
4385/13	65	M	4	N	1,2,5	O			1	A S	N	P	P	A,S
4405/13	64	F	3	N	1,3,5	>3cm			1	A,M				A,A
4445/13	62	M	1	Y	1,3,5	>3cm			1	N PD	P	N	N	F S
4449/13	72	M	4	Y	1,2,5	M,ME	I M		1	S				S
4553/13	56	M	4	Y	1,2,5	>3cm	IM		1	S				S
4562/13	47	M	1	N	1,4,5	<3cm	M	BW-NEG	2	S				S
4679/13	65	F	1	N	1,2,5	H,MNI	M	BW-POS	2	A,M				A,S
476813	45	F	6	N	1,3,4	M,C			1	S				S
4806/13	60	M	1	N	1,3,5	O			1	N PD	N	P	P	F A
4956/13	53	M	1	N	1,4,5	M,C			1	S				S
4972/13	38	F	4	N	1,4,5	M,C			1	S				S
4997/13	67	M	6	Y	1,2,4,5	M,CON	M	BW-NEG	2	S				S
4236/14	57	F	6	N	1,3,5	M	N	BW-POS	1	A M				A S
4237/14	70	M	6	Y	1,2,5	H			1	S PD	P	N	N	S
4327/14	52	M	4	N	1,3,4	O	M	BW-NEG	2	A S	N	P	N	A S
4373/14	42	M	1	Y	1,2,4,5	H	IM		1	S				S
4712/14	71	M	1	Y	4,3,5	M,Rib,V	P		2	N PD	P	N	N	F S
4996/14	54	M	2	Y	1,2,5		M		2	S PD	P	N	N	S
5441/14	63	M	1	N	1,2,5	M			1	N PD	N	N	N	NOS
5489/14	62	M	2	Y	1,4,5	O	M		2	A M				A,A
5527/14	67	M	3	Q	1,5	CAV	M	BW-POS	2	A S	N	P	P	A S
5618/14	77	M	1	Y	1,2,4	H	N	BW -POS	1	S				S
5658/14	67	M	1	N	1,3,5	O	M		2	N PD	N	P	P	F A
5718/14	63	M	4	Y	1,3,5	>3cm,ME	M		2	N PD	N	P	N	F A
5788/14	63	M	4	Y	1,4,5		M		2	S				S
5913/14	58	M	1	N	1,5		M		2	N PD	N	P	P	F A
5995/14	55	M	1	Y	1,3	M			1	N PD	P	P	P	F AS
5997/14	50	M	1	N	1,2,5	M	M	BW-ATYPICAL CELLS	1	N PD	P	N	N	F S
6008/14	65	F	1	Y	1,5		M		2	S				S
6167/14	60	M	4	Q	1,3,4		M		2	N PD	N	P	P	F A
5048/13	54	M	4	N	1,4,5	M,SCN		LN METS	1	S				S
5077/13	58	M	1	Y	1,4,5	M,Rib,PE			1	A M				A S
5080/13	40	F	5	N	1,2,5	M			1	A,M				A,P
5130/13	40	F	1	N	1,2,4	O	M		1	N PD	P	N	N	F S
5166/13	57	M	1	N	1,3,4	O	M		2	A,A				A,A
5341/13	37	M	1	Y	1,3,5	M	M	PF POS	2	A,M				A,MP
5486/13	65	M	1	N	3,4	M	M		2	N PD	N	N	N	NOS
5642/13	70	M	1&2	Y	2,5	M			1	S				S
6050/13	57	M	1	Y	3,4,5	H	M		2	S				S

6052/13	45	F	5	N	1,5	M	M	BW- POS	1	S				S
6223/13	55	M	4	Y	1,3,5	M			1	S				S
6474/13	74	M	1	Y	2,5	H	N	LN METS	1	S				S
6478/13	55	M	3	Y	2,4,5	M	P		2	S				S
6574/13	58	M	4	Y	1,3	H			1	N PD	N	N	N	NOS
6592/13	65	M	4	N	3,5	M			1	A,A				A,A
6618/13	63	M	1	Y	1,2,5	E	M	BW-POS	1	S				S
6679/13	73	M	1	Y	1,4,5	M,Rib,PE	N		1	A,P				A,P
6690/13	52	F	6	N	1,3,5	M	IM		1	A ,M				A,A
6940/13	75	M	1	N	1,4,5	O			1	A,B				A,L
6985/13	45	M	1	Q	1	M			1	S				S
7205/13	72	M	4	N	1,3	M			2	N PD	N	P	P	F A
7253/13	80	F	2	N	1,4,5	M,PE	M	BW-NEG	2	A,M				A,P
7293/13	55	M	4	Y	1,2	M	M	BW-POS	1	S				S
7434/13	53	M	4	Y	1,3,4,5	CON&CAV			4	A M				A,S
7464/13	63	M	1	Y	2,5	>3cm			1	S				S
7475/13	63	M	4	Y	2,4		M		2	S				S
7491/13	75	M	5	Y	1,3,5	M,MNI			1	A M				A,S,M
7715/13	66	M	5	N	1,3,5	F			1	S				S
7830/13	64	M	5	Y	1,2	M	M	BW- POS	2	S				S
7918/13	75	M	5	Y	1,3,4	M,MNI,CAV	M	BW- POS	1	S				S
8074/13	58	M	3	Y	1,4,5	M,MNI,PE			2	S				S
8095/13	49	F	2	N	1,3,5	>3cm,S			1	A,M				A,P
8151/13	45	M	1	N	1,3,5	>3 cm,B	M		2	N PD	N	P	P	F A
8179/13	46	M	4	Y	2,5	>3cm			1	S				S
8218/13	46	M	3	Y	2,4,5	>3cm	NB		1	S				S
8257/13	32	M	2	Y	1,2,5	H,MNI			1	S				S
8317/13	40	F	1	N	1,2	H,MXN	NB		1	S				S
8324/13	67	M	3	Y	1	CON			1	S				S
8525/13	58	M	1	Y	1,2,5	>3cm	M		2	S				S
8539/13	60	F	3	N	1,3,4	M	M		2	S				S
8574/13	60	M	4	Y	2,5	M			1	S				S
8608/13	50	F	5	N	1,3,4	M			1	A,M				A,A
8663/13	60	F	3	N	2,4	H	M		2	S				S
8907/13	70	F	1	N	1,3	M			1	A,P				A,P
8997/13	64	M	1	Y	1,3		NB		2	S				S
9090/13	65	M	4	Y	1,3,4	O	M		1	A M				A,A
9467/13	55	M	1	N	1,4	M			2	S				S
9593/13	54	F	4	N	1,2	M	M		1	S				S
9597/13	55	F	5	Y	1,2,5	>3cm	NB		1	S				S
9780/13	55	M	6	Q	3,4,5	O	M	BW-POS	2	A,M				A,L
9782/13	42	F	5	Q	1,5	M	IM		1	A,B				A,L

9789/13	55	M	6	Y	1,2,3		M		2	N PD				I
9887/13	45	M	1	Q	1,3,5		IM	BW-ATYPICAL CELLS	2	N PD	N	P	P	F A
10024/13	50	F	6	N	1,5	M,C	NA		1	A,B				A,L
10058/13	65	M	1	Y	1,2,4	M,MNI	N		1	S				S
10181/13	45	F	1	Y	2,3	M			1	N PD	P	N	N	F S
10452/13	30	F	4	Y	1,4	M			2	A,M				A,A
10524/13	56	M	4	Y	1,2,4,5	M,MNI			1	S				S
10525/13	42	F	4	N	1,3,5	M	M	BW-POS	1	A M				A P
10555/13	44	M	4	N	3,5	M	M		2	N PD	N	P	P	F A
10569/13	67	M	1	Y	1,3,4,5	M,Rib	NB		1	S				S
10570/13	48	M	4	N	1,2,5	O	M		2	S				S
10650/13	50	M	2	Y	1,3,4,5	M,LI,PE		LN -METS	1	A, B				A,L,M
10664/13	53	M	6	N	1,4,5	M,B			2	N PD	N	P	P	F A
10724/13	34	M	5	Y	1,2	M,CN	P	LN -METS	2	S				S
10763/13	47	M	4	Y	2,4	M			1	S				S
10847/13	60	M	1	N	2,5		M		1	N PD	P	N	N	F S
10882/13	50	F	4	Y	3,4	M			1	N PD	N	P	N	F A
10960/13	60	M	4	Y	2,4		M		2	S				S
10995/1349	49	M	5	Y	1,3	CON	M	SP-POS	2	S				S
11173/13	60	F	6	N	1,4,5	CON,MX N,PE			1	A M				A A
11297/13	60	M	5	Y	1,4,5	>3 cm,F,E	M		1	A M				A A
11300/13	47	M	1	N	1,2,5	<3,CAV,CON	NB		1	S				S
11338/13	45	M	1	Y	1,3		M	BW- POS PF-POS	2	A M				A P
11348/13	67	M	5	Y	1,5	M,L	M	BW-POS	2	S				S
11378/13	45		2&6	N	1,4,5	MX N,B/L,B	M		2	A S	N	P	P	A S
11387/13	75	M	4	Y	1,2	<3cm			1	S				S
11210/13	48	M	3	Y	1,5	M			2	A M	N	P	P	A S

KEY TO MASTER CHART

HPE NO:Histopathological examination number

M : Male

F : Female

Site

1 : Right upper lobe

2 : Right middle lobe

3 : Right lower lobe

4 : Left upper lobe

5 : Left lower lobe

6 : Left hilum

Smoking

Y :Yes

N :No

C/F :Clinical Features

1 : Cough

2 : Hemoptysis

3 : Breathlessness

4 : Chest pain

5 : Weight loss

R/F : Radiological features

M : Mass

H : Hilar mass

ME : Mediastinal invasion

B/L	:	Bilateral lung involvement
C	:	Chest invasion
B	:	Brain metastasis
PE	:	Pleural effusion
CN	:	Cervical node metastasis
MNI	:	Ipsilateral mediastinal lymphnode involvement
MNC	:	Contralateral mediastinal lymphnode involvement
MXN	:	Multiple nodules
O	:	Opacity
F	:	Fibrosis
E	:	Emphysematous changes
A	:	Adrenal metastasis
CON	:	Consolidation
CAV	:	Cavity
LI	:	Liver metastasis
SCN	:	Supraclavicular lymphnode metastasis
RIB	:	Rib erosion
V	:	Vertebral involvement
FOB	:	Fibroptic bronchoscopy
N	:	Normal mucosa
NB	:	Narrowed bronchus
M	:	Mass
P	:	Polypoidal lesion
IM	:	Inflamed mucosa
SP	:	Sputum
BW	:	Bronchial wash
PF	:	Pleural fluid

NEG	:	Negative
POS	:	Positive

Specimen:

1	:	Computed tomogram guided biopsy
2	:	Bronchial biopsy
3	:	Transbronchial bipsy
4	:	Ultrasonogram guided biopsy

HPE diagnosis	:	Histipathological examination diagnosis
A PD	:	Adenocarcinoma poorly differentiated
AS	:	Adenocarcinoma solid type
AA	:	Adenocarcinoma acinar tyype
AB	:	Adenocarcinoma bronchoalveolar type
AM	:	Adenocarcinoma mixed type
AP	:	Adenocarcinoma papillary type
S	:	Squamous cell carcinoma
S PD	:	Squamous cell carcinoma poorly differentiated
N PD	:	Non small cell carcinoma poorly differentiated
AB/PAS	:	Alcian blue/periodic acid Schiff
TTF-1	:	Thyroid transcription factor
P	:	Positive
N	:	Negative
IASLC/ATS/ERS	:	International association for the study of lung cancer/american thoracic society/European respiratory society
AMP	:	Adenocarcinoma micropapillary type
AL	:	Adenocarcinoma lepidic type1